THE EXPLORATORY CLINICAL DEVELOPMENT OF TUCARESOL, AN ANTISICKLING AGENT, USING A NOVEL SURROGATE MARKER.

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CHAPTER 2

THE PHARMACY, PRECLINICAL PHARMACOLOGY,

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APPENDICES - Papers arising from original work presented in this thesis.

Appendix A.

Rolan PE, Parker JE, Gray SJ, Weatherley BC, Ingram J, Leavens W, Wootton R and Posner J (1993).

The pharmacokinetics, tolerability and pharmacodynamics of tucaresol (589C80; 4[2-formyl-3-hydroxyphenoxymethyl]benzoic acid), a potential antisickling agent, following oral administration to healthy volunteers.

British Journal of Clinical Pharmacology 35:419-425.

Appendix B.

Rolan PE, Mercer AJ, Wootton R and Posner J (1995).

Pharmacokinetics and pharmacodynamics of multiple oral doses of tucaresol, an antisickling agent, in healthy volunteers.

British Journal of Clinical Pharmacology 39:375-380.

ABSTRACT

1. Sickle cell disease is a family of inherited haemoglobinopathies resulting from a point mutation in the gene coding for the ß-chain of haemoglobin, resulting in the substitution of valine for glutamate as the sixth amino acid residue on the ß-chain. Sickle haemoglobin (HbS) containing the abnormal ß-chain, functions much like normal haemoglobin (HbA) when oxygenated, but when de-oxygenated, HbS polymerises into helical fibres which distort the normal discoid shape of the red blood cell into a "sickle" shape. It is widely believed that a treatment which prevents polymerisation of deoxy HbS *in vivo* would improve the clinical manifestations of the disease.

2. Tucaresol (4(2-formyl-3-hydroxy-phenoxymethyl)benzoic acid) was designed to bind preferentially to the oxy-conformation of human haemoglobin at a site between the amino terminal residues of the α-subunits, stabilising haemoglobin in the oxy-conformation. This results in a left-shift of the haemoglobin oxygen saturation curve (OSC), increasing the proportion of oxy-Hb at any given low oxygen tension, thereby offering the possibility of preventing sickling *in vivo*.

3. A new surrogate marker (%MOD) had previously been developed to assess the effect of tucaresol and related compounds in man. %MOD is defined as the proportion of haemoglobin molecules reacted with haemoglobin to a high affinity form. It is measured by comparing observed OSC's *ex vivo* with a series of template curves ranging from 0%MOD to 100%MOD in 5% increments. From analysis of the kinetics of formation of the sickle polymer it was estimated that 15 - 30 % MOD would be required for the effective prophylaxis of the manifestations of the disease.

4. This thesis describes the exploratory clinical development of tucaresol, consisting of the three studies performed in man to the date of writing. The first human study with tucaresol was of open, single-dose, ascending-dose, crossover design in 9 healthy male volunteers. Doses ranged from 200 - 3600 mg. Peak concentrations in plasma and erythrocytes were linearly related to dose but were approximately an order of magnitude higher in erythrocytes than in plasma. There was evidence of distribution of drug from plasma to erythrocytes over 24 hours from dosing. Terminal elimination half-life was approximately twice as long from plasma than from erythrocytes, with mean values after the top dose of 289 and 151 h respectively. At the highest dose, peak %MOD was between 19-26%. The drug was well tolerated, with only minor gastrointestinal discomfort at high doses. There were no clear effects on routine haematology and biochemistry, platelet aggregation, resting or exercise heart rates or blood pressures.

5. The second human study was of placebo-controlled, parallel-groups design in 12 healthy male volunteers. The 8 subjects on active drug received three doses of tucaresol at 48 hour intervals. The first was estimated by body weight to achieve 15 % MOD, and the subsequent two doses were individually titrated to produce 25 and 32.5 % MOD. Mean peak achieved %MOD was 34%. Pharmacokinetics were similar to those in the previous study. There was a small increase in heart rate after exercise in the tucaresol group compared to the placebo group. A major unexpected finding was the development of a syndrome of rash, fever and tender cervical lymphadenopathy with onset 7-10 days from dosing suggesting an immune mechanism.

6. The third human study was of double-blind, placebo-controlled, parallel-groups design in 12 stable patients with sickle cell disease. Cumulative doses were progressively reduced from 6400 and 4000 mg over 10 days in the first pair of subjects, to 3000 in the next four patients and 500 mg in the last pair because of rapid rise in haemoglobin and adverse experiences at the higher doses. The pharmacokinetics of tucaresol were similar to those in healthy volunteers, but there was a trend for reduced clearance in women compared to men. Peak % MOD values were 23 and 24%. Three subjects developed fever and tender cervical lymphadenopathy within 7-10 days from the start of dosing. Two were treated with prednisolone with prompt resolution of symptoms. In all six subjects attaining $>10\%$ MOD there was evidence of an antisickling effect of tucaresol, evidenced by rises in haemoglobin, falls in irreversibly sickled cell counts, plasma lactate dehydrogenase and bilirubin.

7. Subsequent *in vitro* and animal studies investigated the possible effects of tucaresol on the immune system. Tucaresol was found to have powerful immunostimulant properties with antiviral and antitumour effects. The likely mechanism was the formation of Schiff's-base adducts with helper T cells mimicking the Schiff's-base mediated communication between antigen-presenting cells and helper T cells. Further evaluation of tucaresol in chronic viral infections and possibly cancer is warranted.

8. This thesis demonstrates that rational drug design may be an efficient way of selecting potential therapeutic candidates. A mechanistically-based surrogate may be very helpful in comparing pharmacology and kinetic studies between animals and man

and help design dosage regimens. However, the clinical pharmacologist in exploratory development needs to look for effects other than those expected.

DECLARATION

The clinical studies described in Chapters 4, 5 and 6, which make up the core of this thesis, were performed by the author within a pharmaceutical company, The Wellcome Foundation Ltd., with extensive resources and many available individuals with specialist skills. Hence all studies required involvement by many individuals and all non-clinical laboratory work was performed by staff other than the author. However, I was the person responsible for:

- evaluating and summarising all available data on the pharmacy, pharmacology, toxicology, mutagenicity, pharmacokinetics and metabolism of tucaresol before the first human study, in order to determine whether a human study was appropriate (this data, almost completely unpublished, is presented in Chapter 2);
- designing the study, writing the protocol, conducting the clinical phase, and interpreting the results of the first human study (Chapter 4);
- designing the study, supervising the preparation of the protocol, conducting the clinical phase and interpreting the results of the second human study (Chapter 5);
- in collaboration with an external investigator, designing the study, writing the protocol, conducting the clinical phase and interpreting the results of the first study in patients (Chapter 6);

and hence this thesis can be said to be my work, except where reference is made to published literature. I also declare that this work has not already been submitted or acceptance in substance for any degree.

I also give permission for a copy of this thesis to be kept in the University library and to be available for loan and photocopying.

Signed, Date....17th Jan 1995

Candidate

This thesis was created using Word Perfect 5.1 (word processing) and Fig P (graphics), running on a Dell Latitude 433CX portable notebook computer, and printed on a Hewlett Packard Laserjet 4 printer with resolution enhancement. The typing was primarily by the author, although some graphics were partially prepared by two of my co-workers, Drs Joe Mercer and Ray Wootton.

DEDICATION

I dedicate this thesis to my wife, Hazel, for her constant encouragement and support.

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- Dr Joe Mercer, Clinical Research Scientist at Wellcome, for writing the protocol, conducting the clinical phase and handling the data, under my supervision, for the study described in Chapter 5;
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GLOSSARY

ACE	angiotensin converting enzyme
ALP	alkaline phosphatase
ALT	alaninine aminotransferase
APC	antigen presenting cell
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUC	area under concentration-time curve
BMT	bone marrow transplant(ation)
bpm	beats per minute
C_e	erythrocyte drug concentration
CI	confidence interval
CL	clearance
C_{max}	maximum concentration
C_p	plasma drug concentration
CPK	creatinine phosphokinase
C_{wb}	whole blood drug concentration
DBP	diastolic blood pressure
DDAVP	desamino d-arginine vasopressin, desmopressin
dP/dt	rate of change of left ventricular pressure
DMA	dimethyladipimate
EEG	electroencephalogram
F	fraction of dose systemically available

H_1, H_2	histamine - 1 or 2 receptor
Hb	haemoglobin
HbA	normal human haemoglobin
HbS	sickle haemoglobin
HBV	hepatitis B virus
HCT	haematocrit
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
iv	intravenous(ly),
k_e	elimination rate constant
LDH	lactate dehydrogenase
LVP	left ventricular pressure
MACL	Mood Adjective Check List
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MHC	major histocompatibility (molecules)
mmHg	millimetres of mercury
%MOD	percent modification of haemoglobin to the high oxygen affinity form
OSC	haemoglobin oxygen-saturation curve
PO_2	partial pressure of oxygen
P_n	pårtial pressure of oxygen for n% saturation of haemoglobin
PT	prothrombin time
RPE	rating of perceived exertion
SBP	systolic blood pressure

SD	standard deviation
TCR	T-cell receptor
Th	helper T lymphocytes
$t_{1/2}$	half-life
t_{max}	time to maximum concentration
V	volume of distribution
V_z	volume of distribution during the terminal elimination phase
5-HT	5-hydroxytryptamine

CHAPTER 1

INTRODUCTION

1.1 **Introduction**

1.1.1 The role of the clinical pharmacologist in exploratory drug development

The full therapeutic utility of a potential new therapy can only be evaluated in adequately powered controlled clinical trials using a suitable reference therapy. However, it is often necessary to obtain pharmacokinetic and pharmacodynamic data in smaller studies before a suitable dosing regimen can be designed which is likely to be optimally effective. These early phases of drug development have traditionally been referred to as Phases I and IIa but for the purposes of this thesis I shall use the term "exploratory development" (Posner, 1994). Until the last decade, the objectives of exploratory development were mainly concentrated on establishing the pharmacokinetic profile of the compound and assessing tolerability. The emphasis on pharmacokinetics is reasonable as in the absence of any other guide to establishing a dosing regimen, half-life and / or residence times may be the best guide to dosing frequency. When Prentis *et al* (1988) reviewed the major reasons for discontinuing drugs in development, the most common single reason was unsuitable pharmacokinetics. Although the pharmacokinetic profile might be useful in establishing dosing frequency on the assumption that it is clinically useful to maintain plasma concentrations within a certain range, suboptimal dosing regimens may be designed using this approach when the assumption is not valid. Pharmacological tolerance or slow transfer of drug between the plasma and effect site might result in inappropriate dosing regimens when these are based on pharmacokinetic principles alone. Examples include the rapid development of clinical tolerance to continuous transdermal nitrates (Frishman, 1992), or the four times daily dosing regimen initially recommended for cimetidine for the treatment of peptic ulcer or ibuprofen for arthritis when less frequent dosing is equally effective.

However even when dosing frequency is in line with the compound's pharmacokinetics, selecting dose size on kinetic grounds alone is usually unlikely to result in the optimum therapeutic dose. Selection of the size of dose for initial evaluation in clinical trials has often been on the basis of the "maximum tolerated" in small studies in healthy volunteers or patients. A major reason for using this approach is to ensure that efficacy will not be underestimated. However this approach has led to excessive doses being used clinically, leading to a false impression of the therapeutic utility of the compound. A good example is the change in therapeutic utility of captopril with reduction in dose. When initially marketed, recommended daily doses were of the order of 150-450 mg. The frequency of significant adverse experiences was such that this drug was initially recommended only for hospital specialist use in patients with resistant medical conditions. However, with an approximate 10-fold reduction in dose, the drug is sufficiently well tolerated to be regarded as suitable for first-line therapy by general practitioners in uncomplicated patients (Brogden *et al*, 1988). One major reason for the initial "overdosage" was the good tolerability of captopril in exploratory studies resulting in the "maximum tolerated" dose being substantially greater than the optimal dose. As a result of the failure of the "maximum tolerated" approach to selecting dose size, drug regulators and prescribers have increasingly insisted that clinical trials explore a dose range below which efficacy is lost but above which efficacy is not increased but adverse effects are likely to be increased, ie the optimal dose range. In clinical conditions in which the therapeutic benefit can be assessed using small numbers of patients in a short period (eg acute treatment of migraine), definition of the optimum dose may most easily be performed by dose-ranging studies. However in conditions in which the therapeutic benefit may only be apparent after long treatment to large groups

of patients (eg prophylaxis of myocardial infarction, arrhythmias, stroke, epilepsy, depression) it may be impractical to design adequately powered clinical studies to compare more than two doses. However, if there were a pharmacodynamic effect, possibly or probably predictive of the desired clinical endpoint, which could be readily measured and which might respond to the drug more quickly than the desired clinical endpoint, initial dosage selection could be based on that effect. Such an effect is often referred to as a *surrogate marker* (Prentice, 1989; Rolan, 1995). The definition of a surrogate is usually defined to a physiologic measurement (Boissel *et al*, 1992) but when it is part of an experimental system it is usually referred to a *model*. Sometimes the distinction is not clear. For example, urinary excretion of leukotriene E_4 is a surrogate for inflammatory activity in asthma; allergen challenge is a model, but measurement of histamine sensitivity could be regarded as either. However, the differences between models and surrogates is operational and not conceptual and hence the concepts can be used interchangeably. A suitable surrogate for captopril would be the inhibition of plasma angiotensin-converting enzyme (ACE), which was the proposed mechanism of action of the compound (Nussberger *et al*, 1987). Clinical dosing of captopril based on ACE inhibition rather than maximum tolerated could have avoided the adverse experiences in some patients.

The utility of a proposed surrogate depends on its validity and practicality. The literature on validation of tests and surrogates is sometimes confusing but there are two principal components of validation of surrogates. The first relatively straightforward aspect consists of obtaining data on the statistical properties relating to reproducibility, accuracy and bias of measurement and may also include observer error and variability

due to assay techniques in addition to biological variation. Some considerations of these statistical aspects are discussed by Prentice (1989) and Boissel *et al*, (1992). The more difficult aspect of validation relates to the information required to support the required assumption that the surrogate shares a causal mechanism with an ultimate clinical outcome. Although a good statistical correlation between the surrogate and the clinical outcome is necessary for this assumption it is not sufficient as the two may have a common root cause but not share a common mechanism. In addition there must be a plausible mechanistic connection between the two and an adequate quantity of experimental data to demonstrate that changes in the surrogate quantitatively predict changes in the clinical outcome after several interventions of different types. Hence validation of a potential surrogate is usually not a binomial variable (valid / invalid) but is a continuous variable. At the beginning of validation of a potential surrogate when little data exists, an estimate can be made on a theoretical basis (a prior probability). As new experimental data becomes available this probability estimate is progressively updated. In accord with Bayes' theorem with the higher the prior estimate of validity the greater the strength of supporting data required to further increase the estimate. This usually means that considerable time and data are required for a surrogate to be regarded as fully validated but a surrogate can be invalidated by a single well-conducted experiment.

Important issues relating the predictive ability of the surrogate (sometimes called *construct* or *criterion* validity) include speed of response to an intervention, sensitivity to the effects of the intervention, and for drug therapies an additional requirement is a dose- or concentration-response relationship. In addition to validation the utility of

a potential surrogate depends on its ease of measurement. Some examples of surrogate markers are listed in Table 1.1.

Table 1.1 Examples of surrogate markers

Compound class	Possible surrogates
H_1-antagonist	Histamine weal and flare in skin (Manna *et al*, 1992); bronchial challenge (Rolan *et al*, 1990)
$5HT_3$-antagonist	Ipecacunaha challenge (Minton *et al*, 1993)
Sedatives	Psychometric tests (Wesnes *et al*, 1987), EEG (Itil *et al*, 1991)
Leukotriene antagonist	Bronchial challenge with leukotriene (Smith *et al*, 1993)
Lipoxygenase inhibitor	5-lipoxygenase inhibitor in neutrophils (Nasser *et al*, 1994); allergen challenge (Weersink *et al*, 1994)
Analgesic	Pain models (Posner *et al*, 1985)
Antisecretory	Gastric pH (Howden *et al*, 1994)
MAO-B inhibitor antidepressants	MAO-B inhibition in platelets, MAO receptor occupancy in brain in vivo (Bench *et al*, 1991)
Antiinfectives	Challenge (eg,malaria, Davis, 1994)

The major factor limiting the utility of using surrogate markers in exploratory development is that the most extensively validated surrogates exist for established therapeutic classes with many available therapies, eg antihypertensives, H_1 and H_2

antagonists. In the exploratory development of compounds with new mechanisms of action it is uncommon for surrogates to exist for which the investigators can be reasonably confident to make decisions on dosing regimen and development strategy, based on data obtained through use of the surrogate. The fastest progress in development of new therapies will come when potential surrogates are measured throughout development of new drugs, including the late phase clinical trials. In this way, validation of the surrogate will progress concurrently with development of the new drug. Although this may not speed up development of the first drug of a series, progressive validation of a potential surrogate may shorten evaluation times of subsequent therapies. Thus a continuous cycle of validation involving evaluation of the prediction of a clinical endpoint from a surrogate and reevaluation, refinement and continued use of the surrogate concurrently with obtaining new clinical information will lead to the most rapid understanding of mechanisms of disease and selection of the most appropriate therapies.

Hence the overall objectives of modern exploratory development have increased to include not only pharmacokinetics and tolerability (assessed by adverse experiences and evidence of organ toxicity) but also to develop, validate and implement the use of surrogate markers to estimate efficacy and to assist in design of dosage regimens.

This thesis describes the exploratory clinical development of tucaresol, under development as an orally administered prophylactic therapy for the symptoms and complications of sickle cell disease. The main clinical consequences of this condition (pain, organ dysfunction) appear intermittently and variably so that long-term clinical

trials would be required to demonstrate clinical benefit. The therapeutic index of tucaresol is also relatively narrow and so dose-escalation studies, in the absence of a good surrogate marker, would have to be cautious and slow. In this thesis, three human studies are described; two in healthy volunteers and one in patients with sickle cell disease. In all three studies, pharmacokinetics and tolerability were examined as well as the relationship between dose, plasma and target tissue (erythrocyte) concentrations of drug and their relationships to a surrogate marker developed for use with this class of compounds. It was hoped that these short term studies (the longest involved 10 day dosing), guided by the surrogate marker, would provide information on which to objectively base a decision to proceed to full clinical development and to select a dosing regimen.

The remainder of this chapter will outline important features of the disease tucaresol was planned to treat and will summarise the rationale for and clinical results with other proposed specific therapies. General supportive therapies, eg analgesics, antibiotics are not discussed although they are important for patient management.

1.2 Sickle Cell Anaemia

Sickle cell anaemia is a homozygous genetic disorder resulting from a point mutation in the gene coding for the ß-chain of haemoglobin resulting in the substitution of valine for glutamate as the sixth amino acid residue on the ß-chain (Ingram, 1957; Dean and Schechter, 1978a). Sickle haemoglobin (HbS), containing the abnormal ß-chain, functions much like normal haemoglobin (HbA) when oxygenated, but when de-oxygenated, HbS polymerises into helical fibres which distort the normal discoid shape

of the erythrocyte into a variety of forms, includung the characteristic "sickle" shape. The rate of sickle polymer formation is highly dependent on the intracellular concentration of deoxy HbS (Sunshine *et al*, 1978). The biochemistry of sickling has been reviewed in detail by Dean and Schechter (1978a and b). The sickled cells are not as easily deformable as normal erythrocytes and it is thought that sickling causes microvascular occlusion which is responsible for the many clinical signs, which can involve almost any organ. Common clinical manifestations include haemolytic anaemia, recurrent episodes of severe pain ("painful crises"), aseptic necrosis of bone, severe bacterial (especially pneumococcal) infections, acute splenic sequestration, delayed growth, progressive renal disease, chronic leg ulcers, priapism and strokes (Smith, 1989).

At present there is no specific therapy and management is based largely on supportive treatment and treatment of complications. However, it is widely believed that a treatment which prevents polymerisation of deoxy HbS *in vivo* would improve the clinical manifestations of the disease. Clinical studies show that the course of sickle cell anaemia is significantly improved by the presence of haemoglobins other than HbS, such as HbA and HbF in erythrocytes and *in vitro* studies confirm that it is the concentration of deoxygenated HbS which determines the rate of sickling (Dean and Schechter, 1978a). Therefore, a treatment which reduces the proportion of deoxy HbS in favour of oxy HbS at low partial pressures of oxygen could be of benefit. This is the proposed mechanism of action of tucaresol which was designed to increase the affinity of haemoglobin for oxygen and thus reduce the concentration of deoxy HbS in erythrocytes in peripheral tissues (Wootton, 1992).

1.3 A review of potential antisickling therapies

Possible mechanisms of action of specific antisickling therapies have been discussed in detail by Dean and Schechter (1978c). These were broadly classified into agents acting at the molecular level (antigelling agents), agents acting at the cell level (cell-sickling inhibitors) and at the vascular level (inhibitors of microvascular entrapment). A modified version of the classification, to incorporate newer therapies, is in Table 1.2. The most important category, to which tucaresol belongs, is that of gelation inhibitors.

Table 1.2 Classification of antisickling drugs

1 Antigelling agents

1.1 Inhibitors of Hb contacts in gel

1.1.1 Non-covalent agents

- urea, alkylureas, guanidine, detergents, organic solutes and solvents, pH manipulation, increasing ionic strength, phenylalanine, aromatic alcohols and acids, 5-bromotryptophan derivatives, propane, ethane, dichloromethane

1.1.2 Covalent agents

- cyanate, glyceraldehyde, pyridoxal sulphate, nitrogen mustard, methyl acetyl phosphate, dimethyladipimate, diaspirins

1.2 Agents which decrease HbS concentration in erythroctytes

1.2.1 Agents which increase erythrocyte water

-DDAVP

1.2.2 Agents which increase HbF

-5-azacytidine, hydroxyurea, erythropoitetin, butyrate and derivatives

1.2.3 Agents which increase Hb oxygen affinity

- cyanate

- valeresol, tucaresol

2 Cell sickling inhibitors

- zinc, danazol, cetiedil, vitamin E

3 Inhibitors of microvascular entrapment

3.1 Vasodilators

-isoxsuprine, co-dergocrine, tolazoline, nifedipine, nitrendipine

3.2 Pentoxiphylline

3.3 Anticoagulants

3.4 Antiplatelet drugs

-aspirin, dipyridamole

These potential therapies are discussed in more detail below.

1.3.1 Gelation inhibitors

Two major approaches have been used in developing specific antigelling agents to reduce the rate of polymerisation of deoxy HbS. These are :

- to disrupt the intermolecular interactions which stabilise the deoxy HbS molecules in a polymer, and
- to reduce the intraerythrocytic concentration of deoxy HbS.

1.3.1.1 Inhibitors of HbS contacts in gel.

1.3.1.1.1 Non-covalent reagents.

A variety of agents which interact non-covalently with haemoglobin to reduce the attractive forces between deoxy HbS molecules has been examined *in vitro* but because of the high concentrations required only a few have been examined *in vivo*. Compounds examined *in vitro* include chaotropic agents such as urea (Allison *et al*, 1957), alkyureas (Elbaum *et al*, 1976), guanidine (Allison *et al*, 1957), detergents (retinol, lysolethicin (Freedman *et al*, 1973)), organic solutes and solvents (Waterman *et al*, 1974, Levine *et al* 1976 a,b). Other approaches have included manipulating pH (Wyman and Allen, 1951) or increasing the ionic strength of the cytosol (Briehl and Ewert, 1973). Several aromatic compounds, including the amino acid phenylalanine and derivatives (Votano *et al*, 1984), aromatic alcohols and acids (Ross and Subramaniam, 1977) and derivatives of 5-bromotryptophan (De Croos *et al*, 1990) were found to increase deoxy HbS solubility. The gases propane and ethane (Milosz and Settle, 1975) and dichloromethane (Schoenborn, 1976) were also found to have antigelling effects *in vitro*. Of all these agents listed, only urea has been studied in man.

1.3.1.1.1.1 Urea

Based on *in vitro* work demonstrating the antisickling effects of urea, a pilot clinical trial (Nalbandian *et al*, 1971) and a multicentre, double-blind, placebo-controlled study were performed using intravenous urea in the management of sickle crisis (Cooperative Urea Trials Group, 1974). The concentrations achieved *in vivo* were far below the 1 mM found to be effective *in vitro* and were limited by dehydration. Although the pilot

study was encouraging, the formal trial demonstrated no benefit and further development of this form of treatment was abandoned.

1.3.1.1.2 Covalent reagents

Agents which covalently bind to haemoglobin to interfere with gelling include cyanate, pyridoxal sulphate (Benesch *et al*, 1974), glyceraldehyde (Benjamin and Manning, 1986; Nigen and Manning, 1977), acetaldehyde (Abraham *et al*, 1982), nitrogen mustard, methyl acetyl phosphate (Ueno *et al*, 1987), dimethyladipimate and diaspirins (Klotz *et al*, 1981; Wootton, 1992), and are reviewed by Ueno *et al* (1989). Except for cyanate and pyridoxal sulphate, these agents have little effect on oxygen affinity. Oral cyanate and extracorporeal cyanate, dimethyladipimate and nitrogen mustard have been studied in man.

1.3.1.1.2.1 Cyanate

Cyanate was initially examined since it was suggested that, as it was produced from urea in solution, it might be responsible for the antisickling effects of urea. Initial *in vitro* studies demonstrated that cyanate markedly and irreversibly reduced morphological sickling of partially deoxygenated erythrocytes (Cerami and Manning, 1971). It was found that cyanate had reacted to produce carbamoylated haemoglobin and this was thought to produce a direct antigelling effect. However, carbamoylated haemoglobin has an increased oxygen affinity which may be the mechanism of action of cyanate (Nigen *et al*, 1973). A pilot clinical study using oral sodium cyanate was encouraging, showing an increased erythrocyte mass and survival, a slight increase in oxygen affinity and a trend for a reduction in the frequency of painful crises (Gillette

et al, 1974). However these findings were not confirmed in a small double-blind crossover study in 17 patients, despite maintaining a mean of 0.48 moles of cyanate per mole of haemoglobin tetramer (Harkness and Roth, 1975). Oral cyanate was also associated with significant clinical toxicity, including reversible peripheral neuropathy and posterior subcapsular cataracts. Because of the lack of efficacy and significant toxicity, further clinical development of oral cyanate was abandoned. However, extracorporeal cyanate was examined in the hope that this could maintain high levels of carbamoylation of haemoglobin and avoid the systemic exposure to free cyanate which was thought to be responsible for the toxicity. Initially, extracorporeal carbamoylation was performed in batches of blood *ex vivo* which were then reinfused into the patient (Deiderich *et al*, 1976). Subsequently, continuous extracorporeal carbamylation was performed with vascular access via an arteriovenous fistula and modified haemodialysis equipment. An initial study in four patients (Balcerzak *et al*, 1982) achieved over 1 mole of cyanate per mole of haemoglobin tetramer without significant toxicity, although one patient withdrew because of severe headaches. In two patients there was a slow increase in haemoglobin levels and falls in reticulocyte counts but there was no trend for clinical improvement in painful crises. Similar results were reported by Lee *et al* (1982) in two patients but a formal clinical trial has not been reported.

1.3.1.1.2.2 Dimethyladipimate

Dimethyladipimate (DMA) is an amino-reactive crosslinking agent which increases oxygen affinity and which shows antisickling properties *in vitro* (Lubin *et al*, 1975). Guis *et al* (1984) reported the results of extracorporeal reaction of blood with DMA

which was then reinfused. Initially there was a four-fold increase in the erythrocyte survival half-life. However, when a second batch of blood was infused 5-7 days later there was rapid destruction of the treated erythrocytes with erythrocyte half-lives of between 1-3 days. This was due to the formation of an antibody to the DMA-treated cells, rendering the treatment ineffective.

1.3.1.1.2.3 Nitrogen mustard

Nitrogen mustard was thought too toxic for systemic administration, despite showing excellent antisickling properties *in vitro*. Roth *et al* (1987) treated erythrocytes extracorporeally with nitrogen mustard and produced increases in erythrocyte survival half-life of between 37 and 62%. No trial of efficacy has been reported.

1.3.1.2 Agents which decrease deoxy HbS concentration.

1.3.1.2.1 Agents which hydrate erythrocytes

1.3.1.2.1.1 Vasopressin and analogues

Water loading was tried as a therapeutic manoeuvre in sickle cell disease since the small reduction in intracellular HbS concentration was thought to be potentially clinically useful. Rosa (1980) reported a study in which three patients received a vasopressin analogue in conjunction with water loading to reduce plasma sodium to 120-125 mM with a parallel decrease in MCHC. Patients acted as their own controls. Reduced *ex vivo* sickling at low partial pressures of oxygen was noted and there was a trend for a reduced frequency of crises. The hyponatraemia was relatively well tolerated provided that plasma sodium was above 120 mM; fatigue and anorexia developed at lower plasma sodium concentrations. However negative results were

reported by Charache and Walker (1981). Despite requiring patients to stay in hospital for 3 months, water loading, sodium restriction and DDAVP administration, persistent hyponatraemia could not be achieved and there was no trend for clinical improvement. A subsequent study by the same group (Charache *et al*, 1983a) examined the effect of hyponatraemia as a treatment for acute crisis rather than maintenance therapy. Eight subjects received active treatment and five placebo. Only one patient appeared to benefit on active drug but when retreated three months later for another crisis, developed no benefit despite sufficient hyponatraemia to produce a convulsion. Hence this treatment, which required intensive blood sampling and electrolyte monitoring, was regarded as impractical and unlikely to offer major benefit.

1.3.1.2.2 Agents which increase HbF

HbF has two γ chains per haemoglobin tetramer instead of the two ß-chains of adult haemoglobin. HbF molecules do not take part in the polymerisation process which occurs during sickling and the rate of polymerisation is inhibited by HbF in a concentration-dependent manner (Noguchi *et al*, 1988). Demonstration of the clinical benefit of an increased proportion of HbF comes from the observation that the clinical severity of sickle cell disease is lessened in patients who also have a hereditary persistence of HbF (Noguchi *et al*, 1988). Furthermore, a low HbF value is associated with increased risk of severe complications of sickle cell disease in children (Bailey *et al*, 1992). A proportion of $>20\%$ HbF or more appears to be required for the beneficial effect of HbF (Noguchi *et al*, 1988) and hence drugs which increase the proportion of HbF might be useful as antisickling agents and several have been or are being evaluated clinically. It is important to keep in mind that the %HbF required to

inhibit sickling with these agents might be much higher than the 20% predicted, particulary early in therapy, because the increased amounts of HbF will not be uniformly distributed throughout the erythrocyte population.

1.3.1.2.2.1 5-azacytidine

5-azacytidine was the first agent to be tested clinically in sickle cell disease with the intention of increasing HbF production. The rationale was based on the observations that 5-azacytidine induced hypomethylation of DNA in tissue culture (Creusot *et al*, 1982) and that DNA is less methylated in tissues where genes are expressed, leading to an expectation that HbF production could be stimulated (Clough *et al* 1982). Initially, increased HbF production was demonstrated in baboons (De Simone *et al*, 1982) and subsequently in a patient with thalassaemia (Ley *et al*, 1982). Two open pilot studies (Charache *et al* 1983b, Ley *et al* 1983) confirmed an increase in HbF production. A subsequent paper reported 4 patients who had received subcutaneous 5-azacytidine for between 30 and 500 days (Dover *et al*, 1985). In three patients there was an increase in F reticulocytes within 24-48 hours and in two patients there was an increase in haemoglobin with little evidence of bone marrow toxicity. One patient was reported to have had no painful crises after 30 days of the study but the authors commented that no conclusions could be drawn from this due to the small number of patients and the open nature of the study.

1.3.1.2.2.2 Hydroxyurea

As 5-azacytidine was considered to be a potential carcinogen, alternatives were sought and Letvin *et al* (1984) demonstrated that another cytotoxic agent, hydroxyurea, also

increased HbF in animals. Further clinical work with 5-azacytidine was not pursued, and hydroxyurea has become the most widely studied specific potential anti-sickling drug. Advantages claimed for hydroxyurea over other cytotoxic agents are that it is relatively nontoxic, its myelosuppressive effects are readily reversible and it is not known to induce secondary malignancies (Goldberg *et al*, 1990). It has been suggested that these agents kill late erythroid precursors leading to recruitment from earlier progenitors that have retained their HbF-producing capacity. However, other mechanisms have also been proposed (Dover *et al*, 1986).

The first publication reporting increased HbF production in man was in 1984 (Platt *et al*) when pulse oral doses were given. Veith *et al* (1985) reported two patients who received pulse dose of hydroxyurea for between 3 days and four weeks. After an initial expected drop in haemoglobin, white cells and platelets, there was a brisk reticulocytosis with the reticulocytes having a marked increase in HbF compared to baseline. A subsequent study in eight patients who received similar pulse treatment produced more initial bone marrow suppression and less subsequent reticulocytosis, with large intersubject variability in response, probably due to varying renal function affecting hydroxyurea clearance (Dover *et al*, 1986). The same group (Charache *et al*, 1987) reported long term results of up to two years open treatment with pulse hydroxyurea in five patients. Using carefully individually titrated doses there were slow increases in HbF from baseline values of 1-3% to 4-18%. The clinical course of two patients was apparently improved. Tolerability was not high; one patient developed megaloblastic anaemia which responded to folate, one developed disseminated zoster, two others had nausea and a further patient was withdrawn from the study after the

first dose because of high fever. As a result of an observation elsewhere that patients with chronic myeloid leukaemia receiving daily hydroxyurea attained higher levels of HbF than patients receiving pulses, hydroxyurea was given daily to patients with sickle cell disease. A careful dose-titration study was performed by Rodgers *et al* (1990) to attempt to define predictors of HbF response. Patients who were resident in hospital for three months were studied. Hydroxyurea was taken four days per week and the dose was slowly escalated from a starting dose based on a measurement of hydroxyurea clearance. Seven patients responded with maximum HbF levels of 10-15% of total Hb. The responses were notably heterogeneous with respect to the pattern and rapidity of response. There was borderline myelosuppression in all patients (including nonresponders) indicating that some degree of myelosuppression may be necessary for an increase in HbF production. No baseline clinical variable appeared to be predictive of clinical response. Four of the seven were retreated for three further months and final HbF levels were 9.4 $\pm$ 2.9%. There were no comments on whether there was any clinical improvement. This study demonstrated that, even under optimum clinical conditions, monotherapy with hydroxyurea achieved levels of HbF that were not quite high enough to produce a worthwhile clinical improvement, required careful clinical monitoring, was not uniformly effective and was accompanied by mild myelosuppression. Furthermore, Dover and Charache (1992) commented in a review on the "dramatic" variation in response and tolerance to hydroxyurea limiting its clinical utility. Further caution was advised by Vichinsky (1994) who reported two patients treated with hydroyurea for between 1 and 4 years. One patient developed an acute painful chest syndrome despite an HbF level of 20% and became pancytopaenic when the dose was increased. The other patient developed a cerebral haemorrhage

despite an HbF level of 21%. These observations also suggest that the therapeutic index of hydroxyurea is low and that major manifestations of sickle cell disease can occur despite apparently satisfactory levels of HbF. In order to determine the clinical utility of hydroxyurea, a major cooperative multicentre placebo-controlled study of hydroxyurea is in progress (Charache, 1994).

1.3.1.2.2.3 Erythropoietin alone and in combination with hydroxyurea.

Stimulation of HbF synthesis by erythropoietin was first reported in baboons by Al-Khatti *et al* (1987). They demonstrated a dose-related increase in F cells within days of dosing with recombinant human erythropoietin. Repeated courses of erythropoietin resulted in approximately 20% of circulating erythrocytes being F-cells. Goldberg *et al* (1990) reported results for five patients who were treated with erythropoietin and hydroxyurea alone and in combination. Initially, patients received very high (up to 1500 U/kg daily for 8 days) iv human recombinant erythropoietin. Three patients later received titrated daily doses of hydroxyurea, and when stable (after about 3 months) high dose erythropoietin was added. Hydroxyurea alone produced a rise in HbF and total Hb, and falls in plasma bilirubin and LDH and a prolongation of erythrocyte half life. However, erythropoietin alone produced no effects on HbF and no additional increase when added to hydroxyurea, despite no evidence of iron insufficiency which would limit the effect of erythropoietin. However, Rodgers *et al* (1993) used a regimen of alternating hydroxyurea (4 days/week) and escalating doses (1000 - 3000 U/kg) of erythropoietin (3 days/week) with daily iron supplementation. The three patients had previously been treated with hydroxyurea and were on individually optimised doses and all had an increase in HbF of between 5 and 18-fold compared to pretreatment. After

erythropoietin, there was a further increase of HbF of an average of 48% and when erythropoietin was discontinued HbF returned to the previous value. During combination therapy there were further falls in plasma bilirubin, LDH and reticulocyte count. Despite these encouraging findings, this proposed therapeutic regimen would be extremely expensive with the very high doses of erythropoietin used and also the haematological toxicity and potential mutagenicity of hydroxyurea make this treatment unattractive, especially for children.

1.3.1.2.2.4 Butyrate

Interest in butyrate for sickle cell disease and thalassaemia arose from the observation that infants who have high plasma levels of α-amino-*n*-butyric acid in the presence of maternal diabetes do not undergo the normal developmental gene switch from the production of predominantly γ-chains to that of ß-chains, without delaying other developmental processes. Butyrate may act through sequences near the start site of transcription to stimulate the activity of the human γ-globin-gene promoter. Perrine *et al* (1993) reported on the use of butyrate in six patients with ß haemoglobinopathies of whom three had homozygous SS disease. They received escalating iv doses of arginine butyrate of between 500-2000 mg/kg/day for periods of 7 days. In the sickle cell patients, the proportion of F reticulocytes increased between 2.2 and 3.6-fold as a result of markedly increased γ-globin synthesis. The infusions were well tolerated but the effect was short-lived and the authors concluded that for this approach to be clinically useful, orally available long half-life derivatives of butyrate allowing longer treatment would need to be developed. Perrine *et al* (1994) have recently reported that

Phase I trials of an orally available butyrate derivative with a long plasma half-life (isobutyramide) have begun.

1.3.1.2.3 Agents which increase oxygen affinity of haemoglobin

An increase in oxygen affinity would result in a reduced proportion of deoxy HbS compared to the oxy-form at tissue oxygen concentrations and hence would reduce the rate of sickle haemoglobin polymer formation. Franklin *et al* (1983) estimated, using carbonmonoxy HbS as a surrogate for oxy HbS, that it would be necessary to maintain 30% of HbS in the oxy-conformation to have a clinically worthwhile antisickling effect. As mentioned in section 1.3.1.1.2.1, cyanate, which has marked *in vitro* antisickling properties, probably acts in part by increasing oxygen affinity in addition to direct inhibition of sickle polymer formation. A number of other agents have been shown to have potential antisickling properties by increasing oxygen affinity and have been reviewed by Wootton (1992). These include pyridoxal phosphate and glyceraldehyde referred to in Section 1.3.1.1.2, as well as valeresol (BW12C) and tucaresol (BWA589C), the subject of this thesis. Apart from cyanate, discussed above, only valeresol and tucaresol, which are discussed in more detail later in this thesis, have been systemically administered to man. Oxygen itself has been studied as a treatment for acute crisis in children (Zipursky *et al*, 1992). Fifteen children received 50% O_2 via a head box and 10 received room air. Although there was a reduction in the reversibly sickled cell count in the active treatment group, there was no difference in the irreversibly sickled cell count, nor was there any trend for clinical benefit from the treatment. The authors hypothesised that the delay in starting treatment (up to 9 days)

was perhaps responsible for the lack of response as tissue damage had already occurred.

1.3.2 Cell-sickling inhibitors

This class of compounds includes those agents which have an antisickling effect through an effect on cell membranes without a direct effect on intracellular polymerisation.

Oral supplementation with zinc was examined by Brewer *et al* (1977) because patients with sickle cell disease were thought to be zinc-deficient and because zinc was thought to have a specific antisickling activity. They found a small decrease in irreversibly sickled cell counts but no clinical results were reported. A beneficial effect was observed, however, in a trial of zinc supplementation in patients with leg ulcers (Serjeant *et al*, 1970).

The local anaesthetic procaine was found to decrease the membrane rigidity of irreversibly sickled cells but a clinical trial has not been reported.

Dietary supplementation with 450 IU of vitamin E for 4 to 35 weeks in 6 patients was reported to reduce the number of circulating ISC's but there was no comment on clinical benefit (Natta *et al*, 1980).

1.3.2.1 Danazol

Danazol, an androgenic drug initially used in the treatment of endometriosis, was studied in patients with sickle cell disease because of a hypothesis that it increased the surface area / volume ratio of erythrocytes and produced some membrane changes. An open prophylactic trial in 5 patients treated for between 7-21 weeks (Temple *et al*, 1986) suggested a benefit with no crises reported in any patient during treatment, a rise in haemoglobin and fall in reticulocyte count in each patient. Despite these encouraging results, further studies do not seem to have been reported.

1.3.2.2 Cetiedil

Cetiedil is a vasodilator which was demonstrated to have antisickling properties *in vitro* associated with membrane effects and not due to an interaction with haemoglobin (Benjamin *et al*, 1980). A subsequent multicenter, double-blind, placebo-controlled, dose-ranging study in 67 patients in acute crisis was reported. There was a statistically significant reduction in pain duration with active treatment in a dose-related manner but the improvement was small (<1 day) (Benjamin *et al*, 1986). Further studies do not seem to have been reported, perhaps due to the lack of impressive clinical benefit.

1.3.3 Inhibitors of microvascular entrapment

1.3.3.1 Vasodilators

Rodgers *et al* (1988) reviewed clinical experience with systemic vasodilators including isoxsuprine, co-dergocrine (Hydergine), magnesium sulphate and tolazoline. All studies reviewed but one were of open design and although positive results were reported, the open design made interpretation difficult. The one placebo-controlled study of isoxsuprine in 40 patients with leg ulcers reported no benefit of active treatment

(Sergeant and Howard, 1977). Rodgers *et al* (1988) hypothesised that vascular obstruction was most likely to occur at the level of the terminal arteriole, the main resistance vessel. Thus they examined the effects of nifedipine, a selective arteriolar vasodilator on the directly observable microvascular beds of the conjunctiva and retina in 11 stable sickle cell patients before and after 20 mg of nifedipine three times daily for 5-10 days. Results were compared with a control group of untreated patients. They found improvements in retinal and conjunctival perfusion, and an improvement in colour vision, in the treated patients as well as falls in plasma bilirubin and haemoglobin, suggestive of reduced haemolysis. They recommended a formal clinical trial but a report of such a study could not be found. No beneficial rheological effects were found in 7 subjects who received 5 weeks treatment with 10 mg twice daily of nitrendipine, a related dihydropyridine calcium antagonist (Nash *et al*, 1991).

1.3.3.2 Pentoxiphylline

Pentoxiphylline is a methylxanthine which has been claimed to decrease whole blood viscosity and improve erythrocyte deformability. Billett *et al* (1989) reported two studies using pentoxiphylline. Initially they examined the effect of pentoxiphylline or placebo in a randomised double-blind study on the course of acute sickle crisis in 16 patients and found no evidence of a benefit with active treatment. Subsequently they conducted an open oral pilot study in 23 stable patients and found no evidence of a therapeutic benefit and concluded that the drug was unlikely to be a useful treatment. Manrique (1987) reported positive results in a prophylactic placebo-controlled, parallel groups study in 60 patients treated with pentoxiphylline 400 mg three times daily or placebo for six weeks. The number of painful episodes, mean pain duration improved

and there were small increases in haemoglobin in the patients on active treatment. However, Teuscher *et al* (1989) reported a randomised placebo-controlled study of iv pentoxiphylline in 36 patients with acute sickle crisis and claimed a statistically significant but modest shortening of pain which was only apparent on day 3.

1.3.3.3 Anticoagulants/antiplatelet drugs

Oral anticoagulation with warfarin was reported by Salvaggio *et al* (1963). Despite a trend for less frequent crises there was a significant increase in bleeding complications. Chaplin *et al* (1980) reported long term observations (2 years) in a partially blinded study in three patients who received twice daily aspirin 650 mg and dipyridamole 50mg. There was a trend for less frequent crises while on active treatment compared to when off treatment. However, daily "low dose" aspirin alone (3-6 mg/kg/day) (Greenberg *et al*, 1983) was of no benefit in a double-blind, placebo controlled, prophylactic study in 49 children, suggesting that platelets do not contribute significantly to the initiation of the vaso-occlusive crisis. This was supported by results from a 6 month placebo-controlled double blind study of ticlopidine in 9 patients which found no trend for therapeutic benefit (Semple *et al*, 1984).

1.3.4 Other treatments

Replacement of erythrocytes containing HbS with ones containing HbA by blood transfusion could be expected to improve the clinical course of sickle cell disease. The utility of transfusion in sickle cell disease has been reviewed by Aluoch (1984) and in more detail by Wayne *et al*, (1993). Tranfusion therapy is administered in one of three modes : acute simple transfusion, chronic simple transfusion, and exchange transfusion.

Transfusion has been part of the manangement of sickle cell disease since 1930 but controlled trials of efficacy are few. Demonstration that prophylactic transfusion can reduce the frequency of crises was provided in a randomised multicentre study in pregnant patients (Koshy *et al*, 1988). In addition to the general complications of transfusion (eg infections, transfusion reactions) there are some specific problems encountered in sickle cell disease (Rao and Patel, 1989; Orlina *et al*, 1991). Hyperviscosity due to excessive transfusion could lead to an increased risk of sickling. The major complication however is iron overload and hence patients receiving chronic transfusion therapy may require iron chelation with desferrioxamine.

An open pilot study of an intravenous infusion of chemically cross-lined bovine haemoglobin in children with acute sickle cell crisis has been reported (Feola *et al*, 1992). The infusions were well tolerated and the authors commented that a major advantage of the solution over conventional transfusion in the country of the study (Zaire) was the high rate of HIV transmission with blood.

A successful bone marrow transplant (BMT) would be expected to offer a "cure" for sickle cell disease. Johnson (1985) reported a child with acute myeloid leukaemia and sickle cell disease who received a BMT. When stable, the levels of haemoglobins A and S were the same as in the donor suggesting the possibility of cure. However, the author stressed the stormy postoperative course and concluded that the utility of transplantation as a treatment for sickle cell disease alone would be extremely limited until immunological management of the transplant is improved. The same author and others (Kodish *et al*, 1990) discussed in detail the dilemma of the trade-off between

early mortality and quality of life, describing the problems of obtaining ethical approval for this form of therapy. Subsequently, the same group reported the results of BMT for sickle cell disease although 2 patients had other indications for BMT (Johnson *et al,* 1994). The complication rate was high with two patients experiencing moderate to severe graft-versus-host disease. The two patients who had had strokes before BMT experienced further neurological deficits. However after a median follow-up of 16 months all pateints were surviving in good to excellent clinical condition with a trend for clinical improvement. Abboud *et al* (1994) reported the results of a successful BMT in a 3-year old girl who had no further crises a year post BMT. Vermylen and Cornu (1994) reported a large European series of 42 patients who had received a BMT as treatment for sickle cell disease. In 36 patients the engraftment was successful and their clinical course was dramatically improved.

1.4 **Discussion**

Despite the large variety of therapeutic approaches and compounds studied for an effect in sickle cell disease, no approach has been particularly successful in producing a practicable and non-toxic treatment. Nonspecific approaches including vasodilation, anticoagulation and antiplatelet drugs have not produced definite clinical benefit. Many specific agent have been too toxic for systemic use at the high concentrations required to produce the desired effect. Extracorporeal treatment, even if effective, would only be available in specialist centres. Cytotoxic agents would always need to be given under close medical supervision and there is some inevitable morbidity associated with their use. Erythropoietin, even if effective is too expensive at current prices to have wide application. Butyrate, which appears promising, requires a suitable prodrug or

analogue to be useful clinically. A need still exists for an orally administered, non-toxic antisickling agent until safe bone marrow transplantation or gene therapy are established clinical practices.

CHAPTER 2

THE PHARMACY, PRECLINICAL PHARMACOLOGY, PHARMACOKINETICS AND TOXICOLOGY OF TUCARESOL

2.1 Introduction

Until the last few decades, the discovery of new drugs has relied largely on serendipity, random searching or the trial-and-error modification of endogenous substances or existing drugs. Recently it has been thought possible that if the full structure of the biochemical target (the receptor or enzyme) is known, it might be possible to design drugs to interact with the target in a desired manner. However, despite the protein sequences of several receptors being discovered, the full quaternary structure and the detailed mechanism of action, which are necessary for this rational drug discovery process, has only been reported for a few macromolecules which are enzymes rather than true receptors. Examples of rational drug design involving the enzymes tetrahydrofolate reductase, carbonic anhydrase and D-ala-D-ala-peptidase are reviexed by Roth and Stammers, (1992). In order to investigate the feasibility of rational drug design by the mechanism of receptor fit, workers at The Wellcome Research Laboratories in the early 1970s designed a series of compounds to interact with haemoglobin, a protein for which the quaternary structure and detailed behaviour were well understood at that time. The discovery of tucaresol and valeresol (discussed in the next chapter) came out of this work, which has been extensively reviewed by Wootton (1992). This chapter reviews the in-house data, mostly unpublished, on the pharmacy, preclinical pharmacology, pharmacokinetics and toxicology of tucaresol which were available before the first human study with tucaresol.

2.2 Physical properties of tucaresol

Chemical name : 4-(2-formyl-3-hydroxy-phenoxymethyl)benzoic acid

Molecular (empirical) formula: $C_{15}H_{12}O_5$

Molecular weight: 272.2

Appearance: colourless crystals.

Melting point: 239-240°C

Solubility: freely soluble in sodium bicarbonate solution

Tucaresol has the following structure :

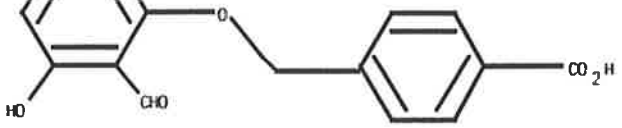


Figure 2.1 Structure of tucaresol

2.3 Biochemistry: interaction with haemoglobin

In the oxy conformation of Hb, tucaresol is believed to interact specifically with the N-terminal amino groups of the two α-chains which are approximately 1 nm apart. The

interaction between tucaresol and the α-terminal region of Hb is made through two linkages (Figure 2.2). On one side of the cleft, a Schiff's base (a "reversible" covalent interaction) is formed between the aldehyde group and one of the α-chain terminal amino groups. This linkage is stabilised by hydrogen bonding with the ortho hydroxyl group. On the other side of the cleft, the carboxyl group is thought to form an electrostatic bond with the other α chain terminal amino group. There is also an important hydrophobic interaction between the aromatic ring and the α-77 proline residues. The overall effect is to constrain the relative orientation of the two α chains and to stabilise the structure of oxygenated Hb. In the deoxy- conformation the two α chain terminal amino groups move to 1.7-1.9 nm apart, and it would not be possible for tucaresol to bind in the same way to this conformation.

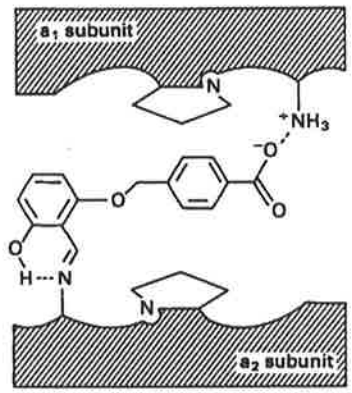


Figure 2.2 Proposed binding of tucaresol to haemoglobin

2.4 Pharmacology

2.4.1 *In vitro / ex vivo*

2.4.1.1 Measurement of Drug Effect

In a haemoglobin solution or erythrocyte exposed to tucaresol in concentrations less than that required to saturate all binding sites, there will be two populations of haemoglobin molecules; those reacted with tucaresol with high oxygen affinity ("modified") and unreacted ("unmodified"). The oxygen saturation curves (OSCs) of the entire solution or population of erythrocytes will thus be a proportional mixture of the OSC's of unmodified and modified molecules. By interpolation between the OSC of 0% modified to 100% modified haemoglobin, it has been possible by computer to construct a set of theoretical curves for varying degrees of % modification which closely approximate to those obtained experimentally using the Hem-O-Scan. This device produces a plot of oxygen saturation as a function of $P0_2$. The percentage of modified haemoglobin (%MOD) can thus be measured in samples by reference to this set of template curves. This technique is quick (approximately 20 min per sample) and has been used extensively in human and preclinical studies with tucaresol and valeresol (Beddell *et al*, 1984; Fitzharris *et al*, 1985; Keidan *et al*, 1986, Nicholls *et al*, 1989a and b). Examples of Hem-O-Scan traces from unmodified, fully modified and partially modified normal and sickle human blood are shown in Figure 2.3.

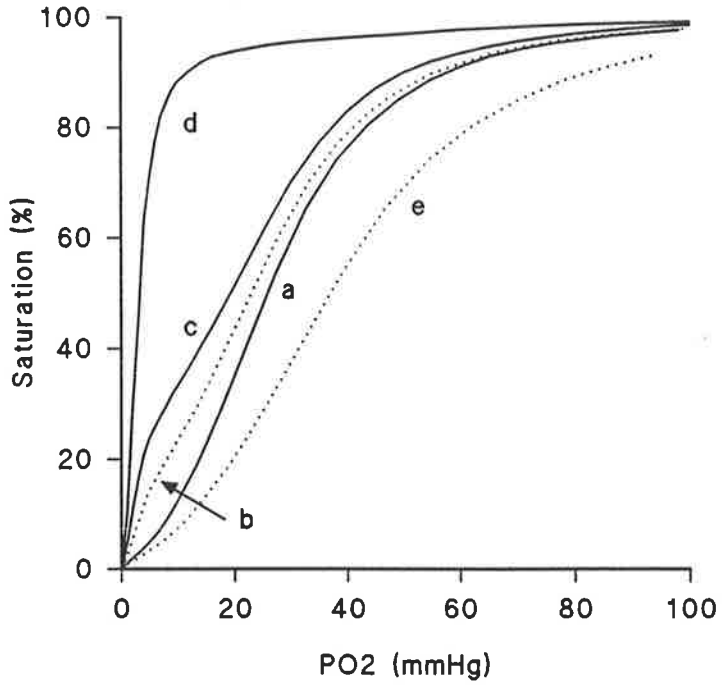


Figure 2.3 Haemoglobin oxygen saturation curves obtained with a Hem-O-Scan

Legend :

a normal human blood, not reacted with tucaresol (0%MOD)

b normal human blood, 15%MOD

c normal human blood, 30%MOD

d normal human blood, fully reacted with tucaresol (100%MOD)

e sickle blood, not reacted with tucaresol (0%MOD)

2.4.1.2 Haemoglobin solutions

The effect of 40 μM tucaresol on the OSC of 23 μM solution of haemoglobin to which 100 μM inositol hexaphosphate (IHP) had been added was examined. The effect of adding IHP was to right-shift the OSC and simulate *in vivo* conditions. In the presence of tucaresol, reacted under oxygenated conditions, there was a pronounced left-shift of

the OSC (ΔP_{20} - 16.6 mmHg), which was similar whether a dissociation or an association curve was being measured. However, if tucaresol was reacted with previously deoxygenated haemoglobin, a different association curve could be measured initially. The interpretation is that whilst tucaresol can react with deoxy haemoglobin and produce an appropriate oxygen association curve, there is preferential binding to the oxygenated conformation and haemoglobin remains locked in the oxy-conformation upon subsequent deoxygenation.

Equilibrium dialysis revealed a very tight 2:1 binding of tucaresol to oxy-Hb in dilute solution. Free tucaresol could not be accurately dialysed and it was not possible to calculate any binding constants. In more concentrated Hb solution more tucaresol was bound giving higher molar ratios and an upper limit to the number of binding sites could not be found.

2.4.1.3 Normal Blood

Normal human whole blood (genotype HbAA, haemoglobin concentration 14 g/100ml) was incubated with various concentrations of tucaresol (0-9 mM), and oxygen saturation curves were measured using a Hem-O-Scan. Tucaresol caused a concentration-dependent left-shift of the OSC (Figure 2.4). Curves at intermediate concentrations were biphasic and the extent of left-shift was dependent upon the proportion of modified haemoglobin which in turn was directly related to the concentration of tucaresol present in the erythrocyte.

Concentrations of tucaresol sufficient to cause a maximal left-shift of the OSC of normal human blood (ca. 3-4 times the molar concentration of Hb) had no effect on several important haematological properties. There was no increased formation of methaemoglobin and no change in the osmotic fragility of the erythrocyte. Erythrocyte Na^+ and K^+ levels were unaffected and there was no change in the Coulter-S profile (mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC)). Tucaresol causes changes in the isoelectric focusing of haemoglobins (normal, sickle, various animals) which are complex and attributable to the net loss of at least two positive charges.

2.4.1.4 Sickle Blood

Sickle blood is right-shifted compared to normal blood due to the presence of deoxygenated polymer and elevated intra-erythrocytic concentrations of 2,3 diphosphoglycerate (2,3 DPG). In experiments similar to those above with normal blood, tucaresol produced a left-shifted OSC comparable to that seen in normal blood. Furthermore, the reduced haematocrit of sickle blood (0.20) results in a greater mole ratio of tucaresol to haemoglobin than in normal blood for a given added concentration. Consequently, tucaresol at a given concentration has a greater effect on the OSC of sickle blood than normal blood even though the nature of the binding to the two Hb genotypes is probably the same.

2.4.1.5 Anti-sickling Action

Suspensions of sickle erythrocytes (haematocrit 0.05) were incubated at 37°C with a range of concentrations of tucaresol (0-0.7 mM) at a partial pressure of oxygen of 30

mmHg, and samples of blood were taken for photomicrography. Erythrocytes were counted on the photomicrographs and were scored as either "normal" (discocyte), "sickle" or "bizarre" (irreversibly sickled cells or echinocytes) and proportions expressed as a percentage of the total count. Tucaresol caused a concentration-dependent inhibition of sickling; the IC_{50} for anti-sickling action being 0.3 mM, and at 0.5 mM the normal cell count was indistinguishable from the aerated control. This experiment was repeated using whole sickle blood and higher concentrations of tucaresol (0-6 mM) in line with the higher haemoglobin content. Sickling was completely inhibited at concentrations of 3 mM tucaresol and above. This concentration of tucaresol results in approximately 50% of the haemoglobin molecules being modified to the high affinity form. This is higher than the 15-30% reduction in deoxy HbS concentration predicted to inhibit sickling *in vivo* (see Chapter 1). However, these experiments were conducted under conditions allowing sufficient time for equilibrium of sickle Hb polymerisation to occur, whereas *in vivo* sickling may need to be delayed only for the duration of the capillary transit time.

2.4.2 Whole animal

2.4.2.1 Conscious rats

2.4.2.1.1 Oral administration.

Groups of 5 male Wistar rats received single oral doses of tucaresol suspended in 0.25% methylcellulose by gavage, at doses of 0, 50, 100, 200, 500, 1000 & 2000 mg/kg.

Behavioural effects. No effects were seen at 50 mg/kg. At 100 mg/kg the only effect observed was flushing of the skin in two rats. At 500 mg/kg, depression of locomotor and respiratory activity 20 - 80 min after dosing was observed in two rats, and ataxia in three. Similar effects but more severe and persisting for up to three days were observed at the higher doses with all animals being affected, but there were no deaths.

Body weight. Rats receiving 1000 and 2000 mg/kg lost weight by 24 h and remained lighter than controls for 8 days after dosing.

Rectal temperature. There were no consistent effects on body temperature.

Gastrointestinal transit. Doses of tucaresol 20 - 200 mg/kg when given orally 1 h prior to a charcoal meal, had no effect on gastrointestinal transit.

Pentobarbitone sleeping time. Doses of tucaresol 20 - 200 mg/kg when given orally 1 h prior to pentobarbitone (25 mg/kg iv) had no effect on sleeping time.

2.4.2.1.2 Intravenous administration.

Groups of 5 rats received single intravenous doses of tucaresol dissolved in sodium hydroxide, diluted as necessary with sodium chloride, at doses of 0, 10, 20, 50, 100, 200 and 500 mg/kg.

Behavioural effects. No effects were seen with the vehicle alone, and the only effect observed at 10 mg/kg was flushing in one rat. No animals were normal at doses of 50

mg/kg and higher with all rats receiving 200 and 500 mg/kg dying between 20 and 80 min after dosing. Death was preceded by depressed activity, poor respiratory effort, loss of reflexes and finally convulsions. Lower doses of 20 - 100 mg/kg induced a slight depression in exploratory behaviour and respiratory rate in half the animals for up to 3 h after dosing, with the degree and duration of these effects being dose related. The marked effects at high doses are probably due to inadequate delivery of oxygen to vital organs as a result of the increased oxygen affinity of modified haemoglobin.

Body weight. No effect was seen at doses up to 100 mg/kg.

Rectal temperature. Tucaresol induced a dose related hypothermia compared with controls for up to 160 min after dosing, with the maximum effect being a decrease of 2 - 8°C in the one rat surviving 200 mg/kg at 40 min after dosing. The lowest dose administered, 10 mg/kg, was without effect.

Gastrointestinal transit. Tucaresol was administered in doses ranging from 5 - 200 mg/kg 10 min prior to a charcoal meal. Doses of 5 and 10 mg/kg were without effect, but at between 20 and 100 mg/kg there was a dose related inhibition of gastrointestinal transit.

Pentobarbitone sleeping time. There was no effect on pentobarbitone sleeping time following doses of tucaresol 5 - 50 mg/kg.

2.4.2.2 Open chest anaesthetised dog

Six anaesthetised dogs received an intravenous infusion of tucaresol of 100 mg/kg over 1 h. Mean %MOD at the end of the infusion was 44% and this level was maintained to the end of the experiment 180 min later.

Heart rate increased during the infusion by 10% and was maintained at this level for the rest of the experiment. There was no significant change in systolic blood pressure, but diastolic blood pressure fell after the infusion had been completed, with the maximum fall of 19% occurring 180 min after the end of the infusion.

There was no significant change in aortic flow or total peripheral resistance, but systolic left ventricular pressure and its rate of change (dP/dt) were increased by 14% and 28% respectively during the infusion and then returned to baseline values after 60 min. Circumflex coronary blood flow increased by 125% and coronary resistance decreased by 53% during the infusion and these changes slowly recovered over the next 180 min. Similar changes have been observed with valeresol (see Chapter 3), an agent with similar action on haemoglobin as tucaresol, in studies in isolated rabbit hearts which demonstrated a decrease in coronary perfusion pressure at constant flow (Allen *et al*, 1986). Other experiments in the blood perfused rat heart using blood with haemoglobin Créteil, a naturally occurring high affinity haemoglobin variant, have also shown increased coronary blood flow (Duvelleroy *et al*, 1980). These observed coronary vasodilator effects may reflect a compensatory response to reduced tissue oxygen delivery per unit volume of blood.

2.4.2.3 Anaesthetised Cat

Tucaresol was administered intravenously over 60 min at doses of 100 and 250 mg/kg to anaesthetised cats. The %MOD achieved at both doses was 100%. This extreme degree of left-shift was not tolerated, resulting in significant tissue hypoxia, necessitating anaerobic metabolism manifested by a severe lactic acidosis. The lower dose of tucaresol caused a slow deterioration in cardiovascular, respiratory, autonomic and neuromuscular function, and the higher dose accelerated these changes with all animals dying within 3 h after the cessation of the infusion.

2.5. Toxicology

2.5.1 Acute i.v

2.5.1.1 Mouse

Single intravenous doses of tucaresol of 0, 89 130 and 168 mg/kg, dissolved in sodium hydroxide solution were administered to groups of 2 male and 2 female mice (except N = 5 for each sex at control dose).

No clinical signs were observed in the control group or at 89 mg/kg. The clinical signs at the higher doses consisted of a slight decrease in activity (moderate in males at 15 min post dose) for up to 5 h post-dosing, together with incidences of trembling, ptosis, unsteady gait and poor righting reflex in both sexes. Additionally, irregular respiration and muscular rigidity were noted in female animals. One female animal, which fitted on injection, died 15 min post injection. Three males developed ulcerated and infected tails (the site of injection) and one was killed on day 3 on humane grounds. However, all surviving animals appeared normal the day after injection and remained so for the

period of observation (14 days). There were no effects on body weight. All organs appeared normal by gross examination at autopsy.

2.5.2 Acute oral

2.5.2.1 Mouse

Groups of 5 male and female mice received single doses of tucaresol by gavage at a dose of 1767 mg/kg suspended in 0.25% aqueous methylcellulose, or vehicle alone, and were observed for up to 14 days. The effects observed were similar in both sexes, with decreased activity, ptosis, poor righting reflex, trembling and muscular rigidity, irregular respiration and piloerection recorded on the day of drug administration in 1 - 3 animals of each sex, with milder effects the following day. Two males and 1 female died within 24 h of dosing. There were no significant effects on body weight.

Abnormal findings at autopsy were confined to animals which died on Day 1, with blood in the skull noted in 2 animals, which was considered to be an agonal change.

2.5.3 Subacute oral

2.5.3.1 Rabbits

Pairs of female non-pregnant rabbits received control or 50, 100, 300 or 500 mg/kg/day of tucaresol orally for 14 days. No adverse effects were seen in the control or 50 & 100 mg/kg/day pairs. In the highest dose pairs severe clinical signs were observed, similar to those seen in the rat, with the dose subsequently being reduced to 400 mg/kg/day from day 3. However, dosing was terminated for this pair on day 7,

with one severely ill rabbit being killed and the other being allowed to recover appearing normal by day 9. Milder effects were seen in the 300 mg/kg/day pair.

Increased red cell counts and haemoglobin were seen at doses of 300 mg/kg/day and above and there was a trend to lower blood glucose levels at 300 mg/kg/day. At autopsy there were no gross or microscopic findings likely to be attributable to treatment.

2.5.4 One month

2.5.4.1 Rat - Dose-range finding study

A preliminary dose-range finding toxicity study was performed in Wistar rats. Groups of 5 male and 5 female rats received tucaresol at doses of 0, 50, 100, 300 and 500 (reducing to 400 - see below) mg/kg/day orally as a single daily dose for 28 days. By analysis of %MOD, doses equivalent to those producing estimated therapeutic concentrations in man are between 100 and 300 mg/kg/day for male rats and between 50 and 100 mg/kg/day for female rats. Blood samples were taken at the end of the study.

Behavioural effects and clinical signs

At low doses (up to 100 mg/kg/day) the only sign noted was pink colouration of the extremities. At 500 mg/kg/day, the rats had severe morbidity including unsteady gait, decreased activity/immobility, with the effect being more marked in the females. Consequently dosing was halted on day 2. Males showed evidence of recovery from these effects and dosing was recommenced at 400 mg/kg/day on day 3 for the

remainder of the study. However, recovery was not seen in the females, and they were killed on day 3.Effects in male rats at 400 mg/kg/day were similar to those seen in females at 300 mg/kg/day, including reddening of the extremities, unsteady gait and abdominal distension. One female at this dose was killed on day 24 due to poor condition.

Haematology

In both sexes and at all dose levels there was a dose related erythrocytosis and reticulocytosis, from control levels of 15.5 g/100 ml and 5.2% (male) and 15.8 g/100 ml and 3.5% (female) respectively to 24.4 g/100 ml and 33% in the male rat and 24.2 g/100 ml and 24% in the females at the highest doses. The erythrocytosis is attributable to the increased oxygen affinity of tucaresol-modified blood, resulting in relative tissue hypoxia stimulating erythropoietin production.

A dose-related leukocytosis was seen in males at all dose levels from a control value of 9.66 x 10^9/l to 14.96 x 10^9/l at the highest dose: in females only the group at 300 mg/kg/day had a significant increase in white cell count at 13.4 x 10^9/l compared to 6.4 x 10^9/l in control. The increased white cell count in both sexes was associated with a decreased percentage of lymphocytes and an increased percentage of neutrophils and monocytes.

In both sexes there was a dose-related decrease in platelet count at all dose levels. In males the platelet count decreased from 1118 x 10^9/l in controls to 328 x 10^9/l at the highest dose, and in females the decrease was from 1056 x 10^9/l to 194 x 10^9/l.

Prothrombin time (PT). In males, there was a small dose-related increase in prothrombin time at all dose levels, although the changes at 50 and 100 mg/kg were not significantly different from control. PT rose from 12.37 s in the controls to 14.18 s at the highest dose. In the females PT was increased only at the highest dose, rising from 12.5 s in controls to 14.23 s at 300 mg/kg/day.

Activated Partial Thromboplastin Time (APTT). In males, APTT rose from 16 s in control to 42 s at 500/400 mg/kg/day. One rat at 300 mg/kg had an APTT of 108 s, 6.7 times the mean control values.

Biochemistry

Plasma glucose was markedly reduced in rats at high doses (control, 7.04 mM; 300 mg/kg/day, 2.48 mM; 500/400 mg/kg/day, 0.78 mM) and to a lesser extent in females (control, 4.64 mM; 300 mg/kg/day, 2.07 mM).

A dose-related increase in aspartate aminotransferase (AST) occurred in males from 100 mg/kg/day upward (control 92 IU/l, 400 mg/kg/day 144 IU/l); a slight increase at 300 mg/kg/day occurred in females. Similar trends were seen in alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) concentrations. Bilirubin was slightly increased in both sexes at the highest dose. Urea and creatinine were slightly increased at the highest dose.

There were no changes in alkaline phosphatase (ALP), albumin, globulin, cholesterol, sodium, potassium, calcium or phosphate concentrations.

Pathology

The weight of spleens and hearts were increased in a dose-related manner. Extramedullary haemopoiesis was noted in the spleens of 2 males at 100 mg/kg/day and all animals at high doses. Hyperplasia of urinary bladder epithelium was noted in 2 rats of each sex at 300 mg/kg/day and all male rats at 500/400 mg/kg/day.

2.5.4.2 Formal one month study in the rat

Another chronic toxicity study was performed in the rat examining lower doses in larger numbers of animals, including a treatment-free period for some rats to assess reversibility of changes. Groups of 18 male and 18 female rats received either control (Group 1) or 50 (Group 2), 100 (Group 3) or 300 mg/kg/day (Group 4) for 1 month. Because of severe clinical signs in the highest dose female group, dosing was withheld on days 3 and 4 and recommenced for the remainder of the experiment at the lower dose of 200 mg/kg/day. Subsequently the only clinical sign noted was reddening of the extremities which was reversible on cessation of treatment. Five animals of each sex and group were autopsied after a 28 day treatment free period. Haematology, biochemistry and %MOD samples were taken at the end of the 28 day treatment period.

Body weight, food intake, water consumption.

There was an initial drop in weight gain in the highest dose male group. There was a small but consistent increase in water consumption in both sexes of Group 4.

Ophthalmoscopy. All Group 4 animals had prominent engorged retinal blood vessels and a generalised red tinge to the retina. At the end of the recovery period these vessels were noted to be only slightly engorged.

Haematology. Haematology variables are summarised in Table 2.1. There was a dose-related increase in the red cell parameters red cell count (RCC), haemoglobin, packed cell volume (PCV), mean corpuscular volume, mean corpuscular haemoglobin (MCH) and reticulocyte count but a decrease in mean corpuscular haemoglobin concentration. These are expected effects, being the haematological response to hypoxia, and were also seen in the previous study. Blood films of the highest dose animals showed slight macrocytosis and polychromasia. There was an increase in white cell count in Group 4 females with an increase in monocyte count and decrease in lymphocyte count. These trends were also present in the dose range finding study but the overall increase in white cell count which was observed in the previous study was not seen in this study. There was a decrease in platelet count in Group 4 males and a dose-related fall in platelet count in females. The reduction in platelet count in high dose animals in both studies may be due to many factors, including decreased production by the marrow or increased destruction due to splenomegaly caused by the drug.

Table 2.1 Mean Haematology data - one month study in the rat

MALES Dose mg/kg/day Group	0 1	50 2	100 3	300 4
Leukocytes x 10^9/l	11.7	11.5	11.8	10.9
% Lymphocytes	77	75	75	69
% Monocytes	2.7	3.9	3.2	6.7
Erythrocytes x 10^{12}/l	7.7	7.7	7.8	8.8
Haemoglobin g/dl	16.0	16.2	16.6	21.2
MCV fl	58.2	58.9	59.7	69.5
MCH pg	21.3	21.4	21.6	24.6
MCHC g/dl	36.2	36.0	35.7	34.9
Platelets x 10^9/l	918	939	919	514
Reticulocytes %	5.4	6.3	6.2	13.9
PT s	17.7	18.1	18.0	20.0
APTT s	21.6	19.8	20.9	21.1

FEMALES Dose mg/kg/day Group	0 1	50 2	100 3	300/200 4
Leukocytes x 10^9/l	8.6	8.7	8.0	10.8
% Lymphocytes	80	76	75	71
% Monocytes	3.3	2.2	3.6	5.2
Erythrocytes x 10^{12}/l	7.5	8.0	8.4	9.2
Haemoglobin g/dl	15.5	16.0	17.9	21.9
MCV fl	58.5	57.7	62	70.7
MCH pg	21.0	20.4	21.6	24.2
MCHC g/dl	35.6	36.0	34.6	33.9
Platelets x 10^9/l	901	842	586	360
Reticulocytes %	3.8	4.1	5.8	17
PT s	18.4	17.4	19.4	22.4
APTT s	19.7	18.9	20.7	20.8

Prothrombin time, Activated Partial Thromboplastin Time. In Group 4 males PT increased by 2.3 s over control with no increase in APTT. In Group 3 females PT and APTT increased by 1 s over control and in Group 4 by 4 and 1.1 s respectively at 47 %MOD and 3 and 4 s respectively at 78 %MOD. These effects on coagulation were not expected but the changes were small at likely therapeutic concentrations. Tucaresol may form Schiff's base adducts with proteins other than haemoglobin (such as coagulation factors), altering their biological action. However, an effect on production of coagulation factors is also possible

Biochemistry. In Group 4 females there was a slight increase in ALT, ALP, and AST. In Group 4 of both sexes there was a slight increase in plasma phosphate and decrease in plasma glucose. No other changes were seen

Urine. No abnormalities were detected.

Organ weight. In Groups 3 of both sexes there was a slight increase in spleen and heart weights. In Group 4 spleen weight was approximately twice control and heart weight was approximately 30-40% increased compared to control. After the recovery period the spleens had reduced to 130% of control and the hearts to 115% of control.

Pathology. Findings at autopsy included general hyperaemia and congestion in the high dose animals. Spleens were increased in size with evidence of extramedullary haemopoiesis. Cardiomegaly was also noted in the high dose animals. Focal and generalised epithelial thickening of the urinary bladder epithelium was noted in Groups 3 & 4 of both sexes. These findings were still present after the recovery period. The

only positive finding on bone marrow examination was a decreased myeloid/erythroid ratio, consistent with increased red cell production.

Effects on the OSC. Table 2.2 shows the range of %MOD in each group of animals from samples taken 6 h post-dose on day 27. By comparison with the data on day 27, no animals were in steady state by day 7 except Group 2 males.

Table 2.2 One month rat toxicology study - ranges of %MOD

Group Dose mg/kg/day	1 0	2 50	3 100	4 300/200
Males %MOD	0	5-9	12-20	34-67
Females %MOD	0	22-25	40-51	73-79

2.5.4.3 Cynomolgus Monkey

Tucaresol was administered orally to groups of males and female Cynomolgus monkeys at a daily dose of 0 (Group 1), 20 (Group 2), 35 (Group 3) and 50 (Group 4) mg/kg/day for 4 weeks, with 2 male and 2 female from the control and high dose groups being allowed an additional 4 week treatment-free period. There were 10 monkeys in Groups 1 and 4 and 6 in the other groups. In Group 4 males mean (SD) %MOD achieved was 36.2 (4.8)% 6 h post-dose on day 8, and 46.6 (5.9)% on day 28, with this %MOD being greater than the expected therapeutic range. However, lower doses produced %MOD in the expected therapeutic range. In females %MOD at each dose was higher than in the males, being 45 (6.8)% in Group 3 6 h post-dose on day 28, and 64 (6)% in Group 4. Thus in females 20 mg/kg/day represents the likely therapeutic range.

Clinical signs - two Group 4 males vomited on isolated occasions. One Group 3 female appeared quiet and huddled during week 3.

Body weight - several Group 4 females lost weight during the first 2 weeks of dosing, associated with a decrease in food intake.

Ophthalmoscopy - no abnormalities were detected.

Electrocardiography - the only abnormality detected was that the QT interval in the Group 4 females was prolonged by 10 msec at the end of treatment when compared to control.

Haematology

Haematology variables are summarised in Table 2.3.

Red cell parameters. Packed cell volume, haemoglobin and red cell count were increased at the end of the treatment period in Group 4 males and Group 3 and 4 females, although the absolute increase was only about 1 g/dl of haemoglobin. There was no reticulocytosis associated with this. At the end of the washout period, the haemoglobin was still slightly further increased. The expected increase in red cells was observed at high doses, but was not observed at likely therapeutic doses.

White cell parameters. In all male groups white cell count was increased. In Group 3 & 4 females there was a decreased white cell count, due to a reduction in neutrophils. The reduction in neutrophil count at high doses seen in the rat was not observed.

Table 2.3 One month monkey study - mean group haematology at end of week 4

MALES Dose mg/kg/day Group	0 1	20 2	35 3	40 4
Erythrocytes x 10^{12}/l	5.5	5.7	5.9	6.2
Haemoglobin g/dl	11.7	12.3	12.3	13.1
PCV %	39.0	40.0	40.0	43.0
MCHC g/dl	30.3	30.8	30.7	30.7
MCV fl	70.0	70.0	67.0	69.0
Reticulocytes %	2.1	2.0	2.0	2.0
Leukocytes x 10^9/l	12.0	8.2	9.5	11.3
Neutrophils %	12.3	22.3	30.7	19.9
Lymphocytes %	85.4	76.5	69.4	78.8
PT s	10.3	9.7	9.8	10.7
APTT s	28.3	27.8	28.7	31.1
Platelets x 10^9/l	359.0	417.0	360.0	318.0
%MOD (6 h post last dose)	0	23.3	32.7	46.6

FEMALES Dose mg/kg/day Group	0 1	20 2	35 3	40 4
Erythrocytes x 10^{12}/l	5.4	5.7	6.3	6.4
Haemoglobin g/dl	11.0	11.6	13.2	13.9
PCV %	37.0	39.0	44.0	45.0
MCHC g/dl	30.1	30.1	30.2	30.7
MCV fl	68.0	68.0	69.0	70.0
Reticulocytes %	2.0	2.0	2.0	2.0
Leukocytes x 10^9/l	11.3	10.2	7.3	6.6
Neutrophils %	40.5	41.3	18.2	21.7
Lymphocytes %	58.9	58.2	77.8	75.8
PT s	10.0	10.1	10.9	11.0
APTT s	30.5	28.1	27.3	42.6
Platelets x 10^9/l	345.0	422.0	378.0	193.0
%MOD (6 h post last dose)	0	22.7	45.3	64.0

Platelets. In Group 4 females, platelet count fell by approximately 50%, and had only partially recovered at the end of the washout period. As in the rat, the reductions in platelet count occurred at high doses but not at likely therapeutic doses.

Coagulation. Prothrombin time increased by approximately 1 s in Group 3 females and Group 4 animals of both sexes. APTT increased by 12.2 s in Group 4 females.

Bone marrow examination (at autopsy). Cell morphology was normal in all films, but lower myeloid: erythroid ratios were seen in some high dose animals of both sexes, probably reflecting the erythrocytosis as a response to the drug.

Biochemistry. There was no significant change in plasma glucose, albumin, globulin, urea, creatinine, alkaline phosphatase, ALT, AST, CPK, bilirubin, sodium, potassium, calcium or phosphate concentrations. There was a slight decrease in cholesterol in all active dose groups.

Urinalysis. No significant changes were seen.

Post-mortem findings. Minimal splenic enlargement was noted in some high dose animals. Heart and spleen weights in Groups 3 & 4 females were increased over control with a similar trend in male in Group 4 males.

2.5.5 Teratogenicity

2.5.5.1 Rat

Groups of 6 female rats received tucaresol, 0, 100, 200 and 300 mg/kg/day on days 6-15 after mating. Effects observed in the mothers were similar to previous studies: unsteadiness alone at 100 mg/kg/day: lethargy, unsteadiness and piloerection at 200 mg/kg/day and severe protracted clinical signs at the highest dose, with 1 animal dying prematurely and the remaining being killed on day 9 on humane grounds. A dose-related erythrocytosis was also observed. No abnormalities were seen in the offspring of 0 & 100 mg/kg/day group, but in the 200 mg/kg/day group there was one foetus with an umbilical hernia/gastroschisis and hooked tail, and another with a reduced/absent eye. Litter outcomes are summarised in Table 2.4.

Table 2.4 Litter Outcomes - rat teratogenicity

Dose mg/kg/day	No of rats pregnant	Pre-implantation loss %	Post-implantation loss %	Mean foetal weight g
0	6	15.2	8.8	3.38
100	5	10.4	4.4	3.44
300	4	14.2	10.5	2.62

2.5.5.2 Rabbit

In an initial study 6 rabbits received tucaresol 0, 100, 200 & 300 mg/kg/day between days 6 - 18 after mating. Behavioural and haematological findings were similar to those from the rat study. There were two total litter losses at 300 mg/kg/day, likely to be due to treatment. The occasional abnormalities detected in

the litters were not attributable to treatment. Embryonic deaths are summarised in Table 2.5.

Table 2.5 Embryonic Deaths - rabbit teratogenicity.

Dose mg/kg/day	Embryonic deaths
0	9.4%
100	5.5%
200	6.1%
300	36.4%

A subsequent study with similar design using doses of 0, 50, 100 & 200 mg/kg/day of tucaresol was conducted using 26 animals at each dose level. In the mothers receiving 200 mg/kg/day, marked and protracted clinical signs were observed including limb rigidity, lethargy and unsteadiness, reduced food consumption and weight gain: 2 deaths and 2 abortions occurred at this dose, thought to be attributable to treatment. Unsteadiness and lethargy occurred at 100 mg/kg/day but there were no maternal effects at 50 mg/kg/day. Foetal weight was significantly reduced in the highest dose group and was slightly lower at 100 mg/kg/day compared with control. A dose-related increase in the incidence of skeletal anomalies was observed, primarily due to disorders of fusion/connection of the sternebrae/costal cartilages. In all actively treated groups there was a slightly higher incidence of foetuses with asymmetric/bipartite sternebrae compared to concurrent

and historical controls. There was also a higher incidence of foetuses with an extra rib or ribs at 200 mg/kg/day and a lower incidence of foetuses with unossified sternebrae at 100 & 200 mg/kg/day. At 200 mg/kg/day there was a high incidence of visceral abnormalities, mainly variations in the origin of the minor arteries from the aorta. Historical controls from the same centre indicate a range of 1-5% for visceral abnormalities. There were no other changes attributable to treatment with tucaresol. The %MOD 6 h following the last dose ranged from 8-12% at 50 mg/kg/day; 20-41% at 100 mg/kg/day and 55-79% at 200 mg/kg/day. Thus, the 50 mg/kg/day received doses closest to those producing the anticipated therapeutic concentrations in man. Foetal abnormalities are summarised in Table 2.6.

Table 2.6 Incidence of abnormalities - rabbit teratogenicity

Dose mg/kg/day	Number examined	Skeletal abnormalities		Visceral abnormalities	
		No.	%	No.	%
0	151	6	4	2	1
50	114	7	6	0	0
100	99	12	12	1	1
200	63	20	32	6	10

2.5.6 Mutagenicity.

Concentrations of tucaresol up to 185 μg/ml were not mutagenic in the Ames test with Yahagi modification.

2.6 Pharmacokinetics

2.6.1 Rat

2.6.1.1 Unchanged drug.

Male and female rats were given 10, 50 & 200 mg/kg tucaresol as a single oral dose, with unchanged drug assayed by HPLC. For both sexes, whole blood elimination half-life increased with dose and apparent oral clearance decreased, suggesting saturation of elimination pathways. The time to maximum plasma and whole blood concentrations (t_{max}) increased with dose, suggesting slower absorption, but this could also be partly due to slower elimination. Females had longer whole blood half-lives and reduced apparent oral clearances than males. The t_{max} in plasma was shorter than t_{max} in whole blood, indicating that uptake of drug by the erythrocytes may be slow. Mean pharmacokinetic values are summarised in Table 2.7.

Table 2.7 Pharmacokinetics of tucaresol in rats - oral administration

Dose mg/kg	Males			Females		
	10	50	200	10	50	200
$t_{max\ plasma}$ (h)	2	2	4	1	2	10
$t_{max\ whole\ blood}$ (h)	4	7.3	7.2	3.5	7.2	11.2
$t1/2_{whole\ blood}$ (h)	5.5	9.2	10.2	9.9	16.1	14.2
$C_{max\ whole\ blood}$ (μg/ml)	22.3	79.4	436	27.9	254	765
CL/F (whole blood)(ml/h/kg)	33.0	28.6	21.0	20.7	9.2	8.8

2.6.1.2 Total radioactivity Following administration of 50 mg/kg of ^{14}C-labelled tucaresol orally and intravenously to male and female rats, urine and faeces were

collected. Recoveries are listed in Table 2.8. Low levels of activity were detected in the carcass and insignificant levels in the expired air. After oral administration the ratio of faecal/urinary excretion was slightly higher in both sexes compared to intravenous administration. In females, the ratio of faecal/urinary recovery was significantly higher and the half life of total radioactivity in whole blood was longer than in males. The longer elimination half-life of total radioactivity from whole blood than of unchanged drug is likely to be due to the presence of metabolites.

Table 2.8 Mean recoveries of total radioactivity (%) - rat

		Male	Female
IV	faeces	41.7	61.6
	urine	45.0	20.24
Oral	faeces	58.0	80.9
	urine	44.3	20.2
$t_{1/2}$ whole blood (h)		28.1	39.3

2.6.1.3 Tissue concentrations

Unchanged drug. Pairs of male and female rats received 50 & 500 mg/kg daily of tucaresol for 14 days. 24 h after the last dose tissue levels of unchanged drug were determined. The tissue with the highest concentration of drug was whole blood. High levels were also found in the gastrointestinal tract. Highly vascular organs such as the liver, heart, lungs, kidney and spleen had levels similar to plasma and very low concentrations were found in the brain.

Whole body autoradiography. Whole body autoradiography was carried out at several time points in male and female albino and pigmented Wistar rats, following an oral dose of 50 mg/kg of ^{14}C-labelled of tucaresol. At all times, highest levels of radioactivity were seen in whole blood and the gastrointestinal tract, with lower concentrations in liver, lung, heart and kidneys.

2.6.2 Rabbit.

4 female rabbits received 50 mg/kg of ^{14}C-labelled tucaresol orally. Mean absorption half life was 1.41 h, and elimination half-life from whole blood was 12.94 h but elimination half-life from plasma was significantly shorter at 6.7 h. Apparent oral clearance and volume of distribution varied widely but in a parallel fashion, resulting in little variability in half-life, suggesting variable bioavailability. The whole blood concentrations of total drug-derived material, determined from radioactivity, were very similar to the concentrations of unchanged drug, suggesting only low concentrations of metabolites in whole blood.

In another 4 female rabbits receiving the same dose, mean recovery of total radioactivity was 80% of which 68% was urinary and 11% was faecal.

2.6.3 Dog

One male and one female beagle each received 30 mg/kg of tucaresol intravenously as the N-methyl-D-glucamine salt daily for 3 days. The half-life of elimination of

unchanged drug was 105 h in the male and 106 h in the female. The maximum %MOD values on day 3 were 40 and 41% respectively and the effect declined with a half-life of 95 h. The elimination half-life in the dog is very much greater than in other species examined.

2.6.4 Monkey

2.6.4.1 Unchanged drug

One male and one female cynomolgus monkey received 3 consecutive daily doses of 30 mg/kg of tucaresol intravenously as the N-methyl-D-glucamine salt.

Volume of distribution (83.4 ml/kg, male; 61.0 ml/kg, female) approximated blood volume. Whole blood clearance was 2.7 ml/kg/h (male) and 1.8 ml/kg/h (female). Elimination half-life from whole blood was 21.6 h (male) and 23.0 h (female). Half-life of decline of %MOD was 34.6 h (male) and 24.5 h (female). The maximum %MOD, achieved 5 min post-dosing on day 3, was 27% in the male and 56% in the female. A good correlation (coefficient = 0.84) was obtained between whole blood concentrations of tucaresol and %MOD.

2.6.4.2 ^{14}C-labelled

Two male and 2 female monkeys received 20 mg/kg of ^{14}C-tucaresol orally and intravenously. After oral administration, t_{max} of total radioactivity was at a mean of 10 h in whole blood and 8 h in plasma. Elimination of total radioactivity was biexponential, with a half-life in whole blood of 19 h during the period 10 - 20 h post drug administration and a half-life of 32 h during the period 120 - 240 h .

Following i.v. administration the half-lives over these periods were 19 and 48 h respectively, agreeing with those of unchanged drug from the previous study. Urinary recovery to 240 h was 59.8% in the urine and 25.0% in faeces.

Radioactivity in plasma and whole blood was due entirely to unchanged drug. Urine contained no unchanged drug, but 2 metabolites. These were also detected in faeces along with an additional metabolite.

As the plasma radioactivity-time curves were similar after oral and intravenous administration (except for the first few hours) and all radioactivity in blood represented unchanged drug, bioavailability appeared to be high.

2.7 Conclusions

- Tucaresol was designed to bind to haemoglobin, preferentially to the oxy-conformation.

- Tucaresol produces a concentration-dependent increase in oxygen affinity of a haemoglobin solution or population of erythrocytes.

- In sickle blood *ex vivo*, tucaresol produces a concentration-dependent inhibition of sickling.

- Acute modification of 40-50 %MOD is accompanied by vasodilation as a compensatory response to maintain tissue oxygen delivery.

- Acute modification to 100 %MOD results in lactic acidosis and death.

- Tucaresol has a moderately narrow therapeutic index.

- Chronic administration of tucaresol produces an expected increase in erythrocyte production but is accompanied by leukocytosis, thrombocytopaenia and impairment of coagulation which were not expected. Apart from these haematological changes there was no other serious toxicity.

- There is a gender difference in tucaresol pharmacokinetics in most species examined which explains the increased sensitivity of female animals in toxicology studies.

- Tucaresol is well absorbed and is eliminated mainly by metabolism but there is little evidence of circulating metabolites.

CHAPTER 3

THE CLINICAL PHARMACOLOGY OF VALERESOL

3.1 Introduction

Valeresol (5-[2-formyl-3-hydroxyphenoxy]pentanoic acid) is a related substituted benzaldehyde which increases the oxygen affinity of haemoglobin in a manner similar to tucaresol (Wootton, 1992). Valeresol was synthesised approximately a year before tucaresol and there was some human experience with it before the first human administration of tucaresol. This chapter summarises the important preclinical differences between the two compounds and the human experience with valeresol; this information was important in the design of the initial human studies with tucaresol.

3.2 Summary of preclinical data of valeresol

3.2.1 Pharmacology

The nature of the interaction of valeresol with haemoglobin is similar to that of tucaresol except that it appears to have a somewhat lower affinity (Wootton, 1992). Cardiovascular pharmacology studies in rats, cats and dogs showed increases in cardiac output and reduction in arterial pressure initially but with cardiovascular collapse and death at doses of approximately 100 mg/kg. There were no significant effects on autonomic, respiratory, central nervous, gastroenterological or renal function until very high intravenous doses had been administered (approximately 100 mg/kg).

3.2.2 Toxicology

Toxicology studies in rats and monkeys showed greater toxicity with oral administration than intravenous administration due to gastric mucosal necrosis.

3.2.3 Pharmacokinetics

Valeresol was extensively metabolised in animals. Whole blood terminal elimination half-life of parent drug varied sigificantly between the species examined with an unusual trend for shorter half-lives in dogs and monkeys than in rats. The mean value in rats was 12 h, in dogs 4.5 h and in cynomolgus monkeys 1.4 h.

3.3 **Human studies with valeresol**

Because of the gastric mucosal toxicity of valeresol this compound has only been administered intravenously to man.

3.3.1 First human study

Before studying the effects of valeresol in patients, it was given to healthy volunteers to examine the kinetics, tolerability and effect on oxygen affinity *in vivo* (Fitzharris *et al*, 1985). Seven healthy male volunteers participated in this open dose-escalating study. Pairs of volunteers received 2, 5, 10 and 15 mg/kg by intravenous infusion and 6 were to receive 20 mg/kg. One infusion at 10 mg/kg was labelled with 17.9 μCi of ^{14}C-labelled drug. The infusions were well tolerated apart from local discomfort at the infusion site which required discontinuation in one volunteer at the highest dose. The ratio of tucaresol concentration in erythrocytes to plasma varied considerably from 1.8 to 26. At 20 mg/kg, mean (SD) $t_{1/2\,\alpha}$ was 2.2 (0.24) h and $t_{1/2\,\beta}$ was 8.4 (1.9) h. Plasma clearance was 0.31 (0.04) ml/min/kg and volume of distribution was 0.067 (0.009) l/kg. Although the data are limited, plasma and erythrocyte AUCs increased greater than proportionally with increasing dose suggesting saturable clearance. In the volunteer who received the radiolabelled

infusion, unchanged valeresol accounted only for a proportion of total radioactivity in whole blood; the proportion declined from 36 % at 15 min from the start of the infusion to 6% at 48 h. In erythrocytes, valeresol accounted for all radioactivity until 15 min after the end of the infusion and the proportion declined to 23% at 12 h after which no further readings were made. These data suggest extensive metabolism of valeresol with metabolites circulating in the plasma but to a lesser extent in erythrocytes. The total recovery of radioactivity was 67% of which 65% was in the urine and 1% each in faeces and the blood samples removed for analysis. At 20 mg/kg, mean (SD) %MOD immediately post-infusion was 15.5 (2.4) %. %MOD declined monoexponentially with a half life of 3.1 (0.5) h.

3.3.2 Study in sickle cell disease patients

In an open study, stable patients with sickle cell disease received valeresol intravenously to examine the kinetics, tolerability and effect on the oxygen saturation curve (Keidan *et al,* 1986) . Initially, the dose was planned to be 20 mg/kg but with a reduction in dose in proportion to the degree of anaemia, as it was thought that sickle cell patients might require less drug than normals to produce the same level of %MOD. Later, patients received the full 20 mg/kg dose without the reduction to take account of anaemia. The infusions were well tolerated. In the 4 patients who received the full dose, peak %MOD at the end of the infusion ranged from 17-23%. There were no consistent trends for changes in haematology profiles or irreversibly sickled cell counts. There was a significant fall in plasma bilirubin (19.3%) and aspartate transaminase (25.3%) at 4 h compared to baseline with a subsequent rise at 24 h, consistent with a reduction in haemolysis.

3.3.3 Effects on moderate graded exercise in healthy volunteers

3.3.3.1 Rationale

Previous studies had shown that acute modification of up to 25 %MOD in volunteers and sickle cell patients was well tolerated at rest. However, it was thought possible that under conditions of increased oxygen demand, tolerability might be impaired. Because sickle cell patients might tolerate a decrease in oxygen delivery less well due to the preexisting anaemia, a study was performed in healthy volunteers to examine the effects of various levels of %MOD on the cardiovascular response to moderate graded exercise.

3.3.3.2 Objectives

To investigate the pharmacokinetics of valeresol at doses producing approximately 20, 30 and 40 %MOD, and to investigate the effects of these doses on the cardiovascular response to graded moderate exercise.

3.3.3.3 Study Design

The study was of open design in 6 healthy male subjects on 3 occasions separated by 14 days. To date, the study has only been reported in abstract form (Nicholls *et al*, 1989a). On the first occasion, valeresol was infused iv at 200 μg/kg/min with the intention of reaching 20 %MOD at 2 h. The infusion rate was then halved to attempt to maintain the level of %MOD for a further 2 h. Target levels of %MOD on occasions 2 and 3 were 30 % and 40 % respectively and infusion rates were adjusted proportionally. Haemoglobin modification was measured at the laboratory half-hourly during the infusion so that the infusion rate could be adjusted to

minimise under- or over-shoot. Moderate graded exercise was performed pre-dose and at 2.25 and 3.75 h after the start of the infusion. Blood samples were taken for assay of valeresol in plasma and whole blood at intervals to 32 h.

3.3.3.4 Methods

Exercise was performed recumbent at 15 degrees from the horizontal on an electrically-braked cycle ergometer, at work rates of 25, 50 and 100 W for 3 min. At the end of each 3 minute period the volunteer was asked to remain still and cardiac output and heart rate recorded by impedance plethysmography (see Chapter 5) over 5 cardiac cycles. Heart rate and cardiac output data were averaged over the second and third exercise tests and were compared to the first test (the pre-drug baseline). At each work rate and for each treatment, mean values and 95 % confidence intervals were calculated for the difference in cardiac output and heart rate post-drug compared to pre-drug. Valeresol was assayed in plasma and whole blood by HPLC.

3.3.3.5 Results

The subjects were of mean age 29 and weight 81 kg. Mean (SD) achieved %MOD on occasion 1 were 18 (2.3) and 22 (2.1) at 2 and 4 h; on occasion 2 values were 28 (1.9) and 32 (4.3) respectively and on occasion 3 values were 37 (2.3) and 41 (2.8) respectively. During the infusions the drug was well tolerated but three subjects consistently reported headaches after stopping the infusions and one subject vomited in the afternoon and evening following the third occasion. The exercise tests were performed without problems. Mean and 95 % confidence intervals for

post-drug differences in heart rate from baseline are presented in Table 3.1. With all treatments, the difference in heart rate from baseline increased with increasing work rate; this was statistically significant at 20 %MOD only at the highest workrate of 100 W; at 30 %MOD significant differences occurred at all exercise levels except at rest and at 40 %MOD significant differences were seen at all exercise levels including at rest. The greatest difference of 17.5 bpm (95% CI 14.5-20.5) occurred at the highest workrate (100 W) at the highest level of haemoglobin modification (40 %MOD). There were no significant differences from baseline in cardiac output.

Table 3.1 Mean and (95% CI) differences in heart rate (bpm) from baseline.

Target %MOD	Exercise level (W)			
	Rest	25	50	100
20	1.0 (-2.1, 4.0)	2.8 (-0.3, 5.8)	2.1 (-0.9, 5.1)	4.7 (1.7, 7.8)
30	0.6 (-2.4, 3.6)	6.9 (3.8, 9.9)	3.9 (0.8, 6.9)	10.1 (7.0, 13.1)
40	4.2 (1.2, 7.2)	11.4 (8.4, 14.4)	12.9 (9.9, 15.9)	17.5 (14.5, 20.5)

3.3.3.6 Discussion

Because of the open design of the study it was not possible to determine whether the high rate of reporting of headache was due to the study procedures (eg caffeine withdrawal) or the study drug. The study demonstrated that higher levels of %MOD than attained previously were acutely well-tolerated at rest and under exercise. Because of the open design and the fixed order of doses, it was not possible to unequivocally attribute the increase in heart rates with dose group to an effect of the drug. However, it was thought that the increases in heart rate were a compensatory response to reduced oxygen delivery per unit volume of blood due to the increase in oxygen affinity. The lack of increase in cardiac output may have been due to the impedance cardiograph not performing satisfactorily under exercise (see Discussion Chapter 5).

3.3.4 Effects on anaerobic threshold in healthy volunteers

3.3.4.1 Rationale

In the previous study the increases in heart rate with increasing %MOD were thought to be due to a compensatory response to decreased oxygen delivery per unit volume of blood. However whether tissue oxygen requirements were being met was not assessed. The good tolerability of the exercise tests may have been due to the relatively low work rates used. A further study was performed to investigate the tolerability of near-maximal exercise under valeresol-induced haemoglobin modification and to examine whether there was evidence of impaired tissue oxygen delivery. This information was thought to be helpful in predicting whether ambulatory treatment with tucaresol would interfere with an active lifestyle in patients.

3.3.4.2 Objectives

To assess the effects of valeresol and the resultant increase in haemoglobin oxygen affinity on the response to exercise in healthy male volunteers with particular reference to lactate production as an indicator of anaerobic metabolism.

3.3.4.3 Study Design

The study was of randomised, balanced, single-blind, 3-period crossover design in 6 healthy male volunteers. To date, the study has only been reported in abstract form (Nicholls *et al*, 1989b). The three treatments were high- and low-dose valeresol and saline placebo. The target levels of %MOD were 40% and 20% and doses of 50 and 25 mg/kg over 2 hours were administered. Before the main study, volunteers performed a graded exercise test producing a peak heart rate of 170 bpm to determine the maximum work rate for each individual in the main study. This level of exercise was the target level in the main study, although the tests were stopped earlier if a heart rate of 170 bpm was achieved at a lower work rate. The exercise test started 10 min after the end of the infusion.

3.3.4.4 Methods

Exercise tests were performed sitting on an electrically-braked cycle ergometer with work rate starting at 0 W and increasing by 20 W each minute until the individual's target work rate had been achieved. Minute ventilation (V_E), carbon dioxide production (V_{CO2}) and oxygen consumption (V_{O2}) were measured using an exercise analysis system (MMC Horizon) which measured gas volume with a turbine meter and infrared and polarographic detectors for CO_2 and O_2 respectively. "Arterialised"

lactate samples were taken from a dorsal hand vein; the hand and lower forearm were enclosed in a perspex cylinder through which warm air at 40°C was blown. Lactate samples were taken at 1 minute intervals during the exercise tests and were assayed using a spectrophotometric technique. The oxygen consumption at a fixed lactate level of 3 mM was taken as an index of anaerobic threshold and values were compared between treatments by analysis of variance.

3.3.4.5 Results

The subjects were of mean age 29 and weight 70 kg. Mean (SD) %MOD at the end of the infusion was 16 (1.7) % after low dose and 32 (4.2) after high dose valeresol: these values were lower than the target levels. Peak work rate tended to decrease with increasing %MOD: mean (SD) values were 213 (10) W, 203 (8) W and 183 (20) W after placebo, low dose and high dose respectively. In the 12th minute, at the end of the test, heart rate was 3.8 bpm (95% CI -4.5, 12.1) higher following low dose compared to placebo, and 17.1 bpm (95% CI 8.8, 25.4) higher following high dose compared to placebo. Although there were no significant changes in respiratory flow, tidal volume or respiratory rate between low dose and placebo, with high dose there was an increased ventilation (mean increase 8.4 l/min; 95% CI 3.1, 25.4) which was due to an increase in respiratory rate (mean increase 3.6 /min; 95% CI 1.3, 5.9) since there was no change in tidal volume. Oxygen consumption at a predetermined lactate level of 3mM was used as a marker of the threshold for anaerobic metabolism. There was no difference oxygen consumption at 3 mM lactate between low dose and placebo, but there was an decrease of 0.24 (95% CI 0.13, 0.35) l/min with high dose.

3.3.4.6 Discussion

This study confirmed the increase in heart rate under exercise compared to placebo of the previous study. The decrease in oxygen consumption for a fixed lactate level is consistent with an impairment of tissue oxygen delivery. However, the absolute values with high dose valeresol were greater than that reported for sedentary individuals (Wasserman and Whipp, 1975). These results suggest that there should be no clinically significant impairment of the ability to conduct normal activities at haemoglobin modification to 30 - 40 %.

3.4 **Conclusions**

- Acute iv administration of valeresol to produce up to 40 %MOD in healthy volunteers and up to 23 %MOD in patients is well tolerated.

- There is an increase in heart rate at rest and under exercise in a dose-related manner which may be a compensatory mechanism to maintain tissue oxygen delivery.

- At high work rates there is increased ventilation and a reduced maximal work rate but the magnitude of these changes is unlikely to be clinically significant.

CHAPTER 4

THE PHARMACOKINETICS, PHARMACODYNAMICS AND TOLERABILITY OF SINGLE ORAL DOSES OF TUCARESOL IN HEALTHY MALE VOLUNTEERS

4.1 Introduction and Objectives

This chapter describes the first administration of tucaresol to man. The first administration to man of a new compound marks an important stage in the progress of a new chemical entity to becoming a useful treatment. It should only be undertaken if there are adequate pharmacology, toxicology and mutagenicity data to allow experienced physicians to assess whether the compound is likely to be well-tolerated in the population studied and if a suitable formulation is available. From the data summarised in Chapter 2, we felt that adequate information was available to proceed to evaluation of tucaresol in man. The specific objectives of this first study were, in healthy male volunteers :

1. To investigate the effects of single escalating doses of tucaresol on:
    - blood pressure, heart rate, respiratory rate, ECG;
    - cardiovascular response to moderate graded exercise on a cycle ergometer;
    - adverse experiences;
    - clinical chemistry, full blood count, coagulation and platelet aggregation;
2. To study the pharmacokinetics in plasma and erythrocytes of single oral doses of tucaresol;
3. To determine the time course of effect of tucaresol on the haemoglobin OSC, and to examine the relationship between these effects and plasma and erythrocyte concentrations.

4.2 Study Design

4.2.1 General

The study was performed according to an open, dose-escalating design involving nine healthy male volunteers. Throughout the study, pharmacodynamic, safety and pharmacokinetic data were examined by the author and other physicians before proceeding to the next dosing occasion. The dosing regimen appears in section 4.3.2.1 - Study Schedule. At intervals throughout each study day the following procedures and measurements were performed:

- blood sampling for determination of %MOD, assay of tucaresol in lysed whole blood and plasma, tests of coagulation and platelet aggregation, full blood counts and clinical chemistry profiles;
- urine collections for urinalysis and assay of tucaresol;
- resting heart rate, systolic and diastolic blood pressure, respiratory rate and ECG;
- heart rate, systolic and diastolic blood pressure and respiratory rate in response to moderate graded exercise on a cycle ergometer;
- symptom enquiry and documentation of adverse experiences.

These procedures and measurements, excluding the tests of platelet aggregation and moderate graded exercise, were also performed at intervals after the study day for a period of up to 96 h. After the study all subjects were examined medically including a full blood count and clinical chemistry profile.

4.2.2 Design Considerations

4.2.2.1 Choice of Study Population

Suitable populations for this study were healthy volunteers or patients with sickle cell disease. Although it is only in patients that the true therapeutic effect could have been assessed, there were several reasons why we preferred healthy volunteers:

- The acute effect of tucaresol to increase the oxygen affinity of haemoglobin could lead to decreased tissue oxygen delivery if not compensated. As patients are already chronically anaemic and with decreased cardiovascular reserve, we thought that they might not tolerate the acute left-shift of the OSC as well as normals;
- We thought that measurement of %MOD would be a good surrogate marker for the desired effect of tucaresol, and a volunteer study would enable us to design a dosage regimen for patients;
- The dose-escalating design required multiple blood samples and the total amount withdrawn might not be as well tolerated in the patients due to their anaemia;
- Patient heterogeneity could have confounded determination of the pharmacokinetics of tucaresol. For example, we expected that a major component of tucaresol distribution would be erythrocyte mass. The varying degrees of anaemia and expansion of blood volume in patients could have made determination of apparent distribution volume difficult. Similarly, varying dégrees of cardiac and renal function might have affected estimates of apparent clearance;

- Many patients receive concomitant drug therapy, such as analgesics (minor and opiate) and non-steroidal anti-inflammatory drugs which might have altered tucaresol pharmacokinetics or response;
- Difficulty in assembling a cohort of adequate numbers of patients who are willing and available to undergo intensive study at the same time.

4.2.2.2 Rationale for Exercise

The expected compensatory responses to the reduced oxygen delivery per unit volume of blood produced by tucaresol include increased tissue blood flow via increased cardiac output. At rest this could be difficult to detect without accurate noninvasive techniques. However, under exercise which increases tissue oxygen demand and cardiac output, we expected that there might be an increase in the heart rate response to exercise, as was observed with valeresol (see Chapter 3). We also felt that absence of a clinically significant effect on exercise in a laboratory would give us more confidence in performing an ambulatory study in patients.

4.2.2.3 Coagulation and platelet aggregation tests

Animal studies had shown inhibition of coagulation (increases in APTT and PT) at high doses producing %MOD in excess of 50%. We thought it prudent to assess *ex vivo* platelet aggregation in addition to APTT and PT.

4.2.2.4 Choice of Doses

Assuming complete bioavailability and that all distribution of tucaresol was limited to erythrocytes it was possible to estimate the maximum possible %MOD as a

function of dose; assuming a haemoglobin concentration of 140 g/l and a blood volume of 5 l haemoglobin mass was estimated at 700 g. Taking the molecular weight of haemoglobin to be 64,500, the haemoglobin mass is 0.0109 moles. From the *in vitro* stoichiometry indicating that between 3 and 4 (average 3.5) moles of tucaresol were required per mole of modified haemoglobin, it was estimated that 1.90 millimoles or approximately 500 mg would be the minimum dose of tucaresol (MW 272) to produce 5% MOD, the limit of quantification of the Hem-O-Scan technique. However, we planned to commence the dose escalation at 200 mg in case there were effects of tucaresol in man not related to haemoglobin modification. Once haemoglobin modification was detected, we planned to escalate the dose arithmetically rather than with the more usual geometric progression, because of the linear relationship between tucaresol concentration and %MOD *in vitro* and *in vivo*.

4.2.2.5. Endpoints for stopping the dose-escalation

We planned to stop the dose escalation at 40% MOD, as this was greater than the expected therapeutic range and animal studies had indicated clinical effects at doses producing %MOD in excess of 50%. The dose-escalation was to be stopped earlier if unacceptable adverse experiences occurred.

4.3 Subjects, Protocol and Methods

4.3.1 Subjects

Subjects were required to be healthy non-smoking men between the ages of 20 and 40 of haemoglobin A phenotype. All volunteers underwent a screening medical

history and examination which included a 12-lead ECG, spirometry, urinalysis (Ames Multistix) and microscopy, full blood count, APTT, PT, clinical chemistry profile, urine screen for drugs of abuse and haemoglobin electrophoresis. Subjects gave written informed consent after a full oral and written explanation of the study and were paid an honorarium on completion of the study.

4.3.2 Protocol

The study protocol was approved by the Wellcome Protocol Review Committee and the Camberwell Health Authority Ethics Committee.

4.3.2.1 Study Schedule

Volunteers were required to abstain from maximal exercise for two days before each dosing occasion and to abstain from alcohol for 24 h before. They fasted from midnight before each dosing occasion but were allowed water *ad libitum*. Pairs of subjects received 200, 400 and 800 mg, and groups of four subjects received 1200, 2000, 2800 and 3600 mg. All doses were given orally with 200 ml water at approximately 8 am following a overnight fast, except for the last two subjects at the highest dose (3600mg - split dose group). In this pair, to avoid gastrointestinal symptoms (see Results - tolerability) 1600 mg was administered at approximately 1 am on the main study day with the remaining 2000 mg at the scheduled time of 8 am, which was still regarded as study time 0 h. Before and at intervals to 24 h post-dose, blood samples were taken through an indwelling venous cannula, with an occluding stylet (Jelco), thus avoiding a heparin lock. Samples were taken for assay of tucaresol (lysed whole blood and plasma), estimation of %MOD, tests of

coagulation and *ex vivo* platelet aggregation, blood counts and biochemistry profiles. Urine samples were taken for urinalysis (Ames Multistix). Heart rate and blood pressure were recorded at intervals and ECG was monitored continuously for the first 24 h. The heart rate response to moderate graded exercise performed on a cycle ergometer was assessed before drug administration and at 4 and 8 h and additionally at 24 h following doses of 2800 mg and above. Meals were provided 5, 10 and 24 h post-dose. Subjects remained in the laboratory for 24 h from dosing. The cannula was then removed and further blood and urine samples were taken at intervals up to 168 h (200 mg), 336 h (400 mg), and 504 h (800 - 3600 mg).

4.3.3 Study Drug

Tucaresol was supplied as 200 and 400 mg tablets by The Wellcome Foundation Ltd., Dartford, Kent.

4.3.4 Laboratory methods

4.3.4.1 Tucaresol Assay in Lysed Whole Blood (Haemolysate)

Whole blood was lysed by the addition of 1 ml of blood to 4 ml of distilled water and the sample was then frozen and analysed subsequently by a sensitive and specific HPLC method.

A 1 ml aliquot of the haemolysate sample was acidified with 500 μl hydrochloric acid, to which was added 50 μl of 12.5 mg/ml internal standard (4[4-formyl-3-hydroxy phenoxymethyl] benzoic acid) (an inactive analogue). 50 μl dimethyl formamide: 0.1% sodium hydroxide carbonate solution (20:80 v/v) was added,

followed by 5.8 ml toluene and mixed for 15 minutes on a horizontal-bed shaker to extract the analytes. Following centrifugation at 500 g, the organic layer was removed and shaken with 1 ml of 0.067 M phosphate buffer (pH 8.5) to back extract the tucaresol and internal standard. A 50 μl aliquot was subjected to HPLC with UV detection at 280 nm. The HPLC system used an octadecyl reversed-phase analytical column at 38°C with a flow rate of 1.4 ml/min. The mobile phase was 63% ammonium acetate (0.1 M):37% acetonitrile (v/v) with 25 ml/l glacial acetic acid and 1.5 ml/l di-n-butylamine. The limit of quantification for tucaresol was 0.5 μg/ml in haemolysate, with a calibration range from 0.5 μg/ml to 50 μg/ml. The bias and precision at 0.5 μg/ml were 15% and 6% and at 50 μg/ml were -1% and 2.5% respectively.

4.3.4.2 Tucaresol Assay in Plasma

A 50 μl aliquot of 12.5 mg/ml internal standard was added to 200 μl plasma, and the sample buffered with 300 μl of ammonium formate / formic acid buffer (0.5M, pH 2.75). The sample was then microcentrifuged and the analytes adsorbed onto a preconditioned solid-phase extraction cartridge (C18, 100 mg) and washed with two consecutive 1 ml buffer washes and 1 ml of water. The analytes were then eluted with 250 μl of methanol and an aliquot was analysed by the same HPLC system as for haemolysate. The limit of quantification was 0.5 μg/ml with a calibration range from 0.2 μg/ml to 40 μg/ml. Bias and precision were -4% and 7 % at 0.5 μg/ml and 1% and 4% at 40 μg/ml respectively.

4.3.4.3 Haemoglobin modification - %MOD

0.5 ml of whole blood was kept at 4°C and the haemoglobin-oxygen saturation curve was determined within 24 h using a Hem-O-Scan apparatus (Beddell *et al*, 1984). Haemoglobin modification less than 5% cannot be reliably measured and was recorded as less than 5%.

4.3.4.4. Haematology

Erythrocyte, white cell and platelet counts were determined with a Coulter Counter. Reticulocyte counts and differential white cell counts were performed by manual staining methods. Activated partial thromboplastin times and prothrombin times were determined using standard methods.

4.3.4.5 Platelet Aggregation

Ex vivo platelet aggregation was assayed in citrated whole blood. Fixed amounts of aggregating agents are added (adenosine diphosphate, 2 μM and collagen, 0.5 μg/ml) to produce an approximately 90% acute fall in the count of single platelets. Platelet counts were performed with an Ultraflo 100 Whole Blood Platelet Counter. Inhibition of platelet aggregation appears as a reduced initial fall in the count following the addition of the aggregating agent and a faster subsequent rise (Lumley & Humphrey, 1988).

4.3.5 Clinical Measurements

4.3.4.1 Exercise Test

Heart rate at rest and systolic and diastolic blood pressures were determined with an automated oscillometric device (Hewlett Packard 78354A). Respiratory rate was

measured by observation for 30 s. The exercise test was performed supine at 7.5° from the horizontal on an electrically-braked cycle ergometer (Lode). Exercise was performed at work rates of 40, 80 and 120 W for 3 min at each level, with an interval of 1 min between levels. Heart rate was measured at the end of each exercise period from a 5-beat portion of the monitored ECG tracing.

4.3.6 Data Analysis

4.3.5.1 Pharmacokinetics

Erythrocyte concentrations of tucaresol C_e were calculated as follows:

Amount of drug in unit volume of whole blood = amount of drug in plasma + amount of drug in erythrocytes.

$$\Rightarrow C_{wb} = C_p.(1 - Hct) + C_e.Hct$$

$$\Rightarrow C_e = \frac{C_{wb} - (1 - Hct).C_p}{Hct} \qquad \textbf{Eqn 4.1}$$

where C_{wb} and C_p represent tucaresol concentration in whole blood and plasma respectively, and Hct represents the haematocrit.

Concentrations were subjected to pharmacokinetic analysis by standard non-compartmental methods using Siphar, (Simed, Creteil, France). Terminal elimination rate constant (k_e) was determined by log-linear least-squares regression analysis of the terminal segment of the log concentration - time plot. $AUC_{0-\infty}$ was calculated as $AUC_{0-\infty} = AUC_{0-t} + C_t/k_e$, where AUC_{0-t} was calculated by the linear trapezoidal rule, and C_t was the concentration at the last time point, t. CL/F

was calculated as Dose/$AUC_{0-\infty}$, and V_z/F as $CL/(F.k_e)$. C_{max} was the maximum observed concentration and t_{max} the time it occurred.

4.3.5.2 Statistics

As the study was of an exploratory nature, means and standard deviations were primarily used to describe data at each dose, except at the three lowest doses when individual results are presented as only two subjects were studied. 95% confidence intervals were calculated for the individual erythrocyte / plasma ratios for C_{max}, AUC_{0-inf}, and $t_{1/2}$. The Pearson correlation coefficient was used to test for dose-dependency of pharmacokinetic variables with p values from the appropriate T statistic. Linear regression was used to quantify the relationships. Medians were calculated for t_{max} and a 95% non-parametric confidence interval was calculated for the difference in erythrocyte and plasma t_{max} using the Wilcoxon signed-rank test.

4.4 **Results**

4.4.1 Volunteer accountability and demographics

Volunteer demographics are summarised in Table 4.1. Initially eight volunteers were recruited. One subject (no. 5) completed only one occasion and was withdrawn due to adverse experiences as described below (see section 4.4.4). Subject 9 was recruited as a replacement.

4.4.2 Pharmacokinetics

Pharmacokinetic variables are summarised in Table 4.2. The mean concentration-time curves of tucaresol in plasma and erythrocytes following 2800 mg tucaresol are

shown in Figure 4.1. C_{max} values in plasma and erythrocytes were proportional to dose. Mean C_{max} in plasma at the highest dose of 3600 mg was 95.8 μg/ml and in erythrocytes was 1035 μg/ml ; there appeared to be no difference between the single dose and split dose pairs. The ratio of erythrocyte C_{max}/plasma C_{max} increased with dose ($r=0.80$; $p<0.001$) from 3.8 and 4.4 at 200 mg to a mean (SD) of 11.2 (1.9) at 3600 mg (Figure 4.2). t_{max} was earlier in plasma (overall median 6.5 h, 3600 mg split dose pair not included) than in erythrocytes (median 24.5 h); (overall median and 95% non-parametric CI for difference : 18.5 h, 15 - 29 h). Plasma concentrations fell biexponentially with an initial fall between t_{max} and approximately 24 h and a slower terminal phase subsequently, but erythrocyte concentrations fell monoexponentially. The terminal half-life from erythrocytes (mean 151 h at 3600 mg) was shorter than that from plasma (mean 289 h); overall mean ratio for erythrocyte $t_{1/2}$ / plasma $t_{1/2}$ was 0.57 (95%CI; 0.53 - 0.61). Both half-lives increased with dose ($r=0.77, <0.0001$ for plasma; $r=0.51$, $p<0.05$ for erythrocyte; Figures 4.3 and 4.4). k_e and hence half-life and $AUC_{0-\infty}$ could not be reliably calculated following 200 mg because the sampling interval was only approximately one half-life. Apparent clearance was independent of dose. $AUC_{0-\infty}$ was higher in erythrocytes than in plasma and the erythrocyte / plasma ratio increased with dose ($r=0.75$, $p<.0001$; Figure 4.5).

No formal analysis of tucaresol concentrations in urine was performed as the assay had not been fully validated. However, using a modification of the assay for plasma tucaresol was detected and an estimate of the recovery of unchanged drug in the urine from one subject was less than 1%.

4.4.3 Haemoglobin Modification - %MOD

Individual values of peak %MOD are given in Table 4.2. Haemoglobin modification was not detected at doses of 800 mg and below. Peak %MOD following 3600 mg ranged from 19 - 26% and occurred at a similar time as maximum concentrations in whole blood. The %MOD - time curves were parallel with the erythrocyte tucaresol - time curves (Figure 4.1). In all subjects %MOD returned to less than 5% by the end of the sampling period. The relationship between %MOD and the molar ratio of erythrocyte tucaresol to haemoglobin ratio (Figure 4.6) was linear without evidence of hysteresis for individual subjects.

4.4.4 Tolerability and Adverse Experiences

Overall the drug was well tolerated. Subject 5 who received 1200 mg developed a headache and vomited approximately 10 h following drug ingestion and was withdrawn. The first two subjects who took 3600 mg reported slight abdominal discomfort and loose stools. For the remaining two subjects the dose was split into two fractions of 1600 and 2000 mg separated by 7 h and there were no further reports of gastrointestinal symptoms. Full details of adverse experiences are listed in Table 4.3.

4.4.5 Haematology

There were no trends for changes in the erythrocyte, leukocyte or platelet numbers or for erythrocyte haemoglobin F content throughout the study. Three of the four volunteers receiving 2800 mg had an elevation in reticulocyte count above the

reference range (<2%) to peaks of 2.2 - 3.1% between days 7 and 14 (Figure 4.7). Two of the four subjects receiving 3600 mg had peaks of 2.1 and 2.8% between days 14 and 28 (Figure 4.8). No changes in coagulation or platelet aggregation were observed. The mean platelet aggregation data at 2800 mg are shown in Figures 4.9 and 4.10.

4.4.6 Cardiovascular Parameters

There were no trends for changes in resting heart rate or blood pressure and no differences in the exercise-induced heart rates between pre-drug and 24 h post-drug (Figures 4.11, 4.12 and 4.13). There were no changes on ECG.

4.4.7 Other Tests

There were no significant abnormalities of clinical biochemistry or urinalysis.

4.5 **Discussion**

Tucaresol is an unusual drug in that blood sampling allows measurement of plasma concentrations as well as target tissue concentrations (erythrocyte). The pharmacokinetics of tucaresol are complex. Plasma concentrations peak earlier than in erythrocyte. Within the first 24 h plasma concentrations fall while erythrocyte concentrations are rising, indicating that there is redistribution of drug from plasma to erythrocyte during this time. At the time of peak whole blood concentrations following 3600 mg, approximately 70% of the administered dose is present in blood, assuming a blood volume of approximately 5 l. This indicates that bioavailability is high. Similarly, from peak erythrocyte concentrations and an

average haematocrit of 0.42, approximately 60% of the administered dose is in the target tissue of the erythrocyte.

Elimination half-life from plasma was significantly longer than that from erythrocytes. If drug in plasma and erythrocytes were in dynamic equilibrium during the apparent terminal phase the half-lives should be similar. A possible explanation for this difference is that some drug in plasma may be functionally in a "deep" compartment. This could occur if some drug is bound very tightly to plasma proteins with a long half-time of dissociation *in vivo*. The formation of a Schiff's base adduct between the aldehyde group of tucaresol and amino groups of proteins could result in a "reversible" covalent reaction with high stability. However, this interaction would need to be reversible on acidification by the extraction procedure for HPLC for the drug content to be measured. Human plasma protein binding *in vitro* was >98.5% and independent of concentration over a range exceeding the peak concentrations in this study. However, at present the proteins to which tucaresol binds in plasma and the nature of the interaction are unknown.

As dose increased, the ratio of $AUC_{0-\infty}$ in erythrocytes to plasma increased showing that a greater proportion of drug in blood is in erythrocytes compared to plasma as dose increases. This results in an increasing half-life with dose, as plasma clearance is unaffected. It is unlikely that this change in distribution is due to decreased plasma protein binding from the *in vitro* results mentioned above, and the lack of

change in clearance which should be binding-sensitive. The mechanism of this change in distribution is therefore not clear.

The elimination half-life of tucaresol from whole blood of approximately one week suggests that tucaresol is suitable for chronic use in the prophylaxis of the clinical consequences of sickle cell disease. There is evidence that the likelihood of painful crises in a patient with sickle cell disease increases with haematocrit (Baum et al, 1987). Chronic administration of tucaresol may result in a significant increase in haematocrit due to an inhibition of sickling and stimulation of erythropoiesis. Sudden cessation of an anti-sickling effect could thus result in a greater risk of a painful crisis than before treatment. However the long half-life of tucaresol may well prevent such problems even if drug administration were abruptly discontinued.

This study demonstrated that administration of tucaresol sufficient to modify 19 to 26% of haemoglobin to a high affinity form was well tolerated by these healthy volunteers. This is consistent with the lack of clinical consequences in people with naturally occurring high affinity haemoglobinopathies, with the exception of polycythaemia. Polycythaemia, an expected response by the bone marrow to a hypoxic stimulus, was observed in animals treated chronically with high doses of tucaresol (see Chapter 2). The slight increase in reticulocyte count in this study, insufficient to increase haematocrit, may be a threshold effect of tucaresol on erythropoiesis.

The increased oxygen affinity of haemoglobin modified with tucaresol could reduce the tissue oxygen delivery per unit of volume of blood. Oxygen delivery could be maintained by increasing blood flow. Evidence of coronary and systemic vasodilation was observed in anaesthetised dogs following intravenous administration of tucaresol to produce a mean peak 44%MOD (see Chapter 2). In healthy volunteers receiving intravenous valeresol in doses producing 20, 30 and 40%MOD over 2 h, there was a dose-related increase in the heart rate response to moderate exercise (see Chapter 2, Nicholls *et al*, 1989a). The lack of clear effect on the exercise heart rate in this study is probably due to the lower peak %MOD, and perhaps the relatively longer time from baseline to peak %MOD.

The linear relationship between %MOD and the molar concentration ratio of erythrocyte tucaresol to haemoglobin is in agreement with the relationship obtained *in vitro* (Beddell *et al*, 1984; Keidan *et al*, 1989). The slope of 0.26 is also in agreement with the *in vitro* data indicating that 3 - 4 moles of tucaresol are required per mole to fully left-shift the haemoglobin tetramer.

The linear relationship between %MOD and whole blood drug levels suggests that if facilities to measure %MOD by Hem-O-Scan are unavailable for clinical monitoring, (as the equipment is now out of production) measurement of the whole blood drug concentration may suffice. This would need to be validated in a clinical setting in patients with sickle cell disease and with a wider range of haematocrits than the healthy population used in this study.

In this dose-escalating study of tucaresol in healthy volunteers, no adverse experiences or laboratory abnormalities were reported of sufficient importance to preclude further development.

4.6 Conclusions

- Tucaresol is well absorbed orally with a bioavailability of at least 70%.

- After absorption there is redistribution of drug from plasma to erythrocytes.

- Elimination half life in man is considerably longer than in the animals in which the main toxicity studies were performed.

- Elimination half life from plasma is longer than that from erythrocytes perhaps due to tight binding to a plasma component.

- Acute oral administration of up to 3600 mg of tucaresol producing up to 26 %MOD was well tolerated symptomatically and haemodynamically with the exception of minor gastrointestinal symptoms at single doses over 2000 mg.

- Tucaresol is suitable for further clinical development as an antisickling agent.

TABLES

Table 4.1 Volunteer demographics and doses

Subject No.	Age	Sex	Weight (kg)	Height (m)	Doses (mg)
1	30	M	82.5	1.85	200, 1200, 2800
2	31	M	77.8	1.81	200, 2000, 3600
3	34	M	65	1.76	400, 1200, 2800
4	25	M	69	1.74	400, 2000
5	33	M	80.5	1.86	1200
6	28	M	74	1.78	1200, 2000, 3600
7	23	M	84.7	1.77	800, 2800, 3600
8	38	M	68	1.79	800, 2800, 3600
9	35	M	82	1.73	2000
Mean	30.8	-	75.9	1.79	-
SD	4.8	-	7.2	0.05	-
Minimum	23	-	65	1.73	200
Maximum	38	-	84.7	1.86	3600

Table 4.2 Summary of pharmacokinetic and %MOD data

Subject No	Dose (mg)	Plasma						Erythrocyte					%MOD			
		k_e h^{-1}	$t_{1/2}$ h	CL/F ml min^{-1}	C_{max} $\mu g\ ml^{-1}$	t_{max} h	$AUC_{0-\infty}$ $h.\mu g\ ml^{-1}$	k_e h^{-1}	$t_{1/2}$ h	C_{max} $\mu g\ ml^{-1}$	t_{max} h	$AUC_{0-\infty}$ $h.\mu g\ ml^{-1}$	k_e h^{-1}	$t_{1/2}$ h	Maximum %MOD	t_{max} h
1	200				9.9	5				37	10					
2					11.1	5				49	10					
3	400	0.00522	133	2.35	16.6	4	2829	0.00762	91	82	12	13214				
4		0.00365	190	1.86	19.6	3	3562	0.00553	125	115	24	29863				
7	800	0.00516	134	2.72	28.3	4	4889	0.00820	85	173	24	29573				
8		0.00549	126	2.31	36.5	4	5759	0.00791	88	241	16	35011				
5	1200	0.00275	252	1.65	40.3	8	12103	0.00395	176	339	24	91181			7	12
6		0.00361	192	2.17	40.1	6	9180	0.00601	115	306	48	72449			7	8
1		0.00564	123	3.33	39.3	4	5987	0.00899	77	244	24	38739			5	6
3		0.00323	215	2.44	33.7	8	8161	0.00695	100	306	51	55235			7	12
Mean		0.00381	194	2.40	38.4	6.5	8858	0.00648	117	299	36.7	64401			6.5	9.5
2	2000	0.00250	278	2.04	60.8	6	16273	0.00501	138	671	24	169144	0.00323	215	17	49
4		0.00312	222	2.01	59.3	6	16487	0.00640	108	641	24	191881	0.00333	208	16	24
6		0.00335	207	2.37	57.1	6	14010	0.00526	132	650	50	147352	0.00778	89	12	24
9		0.00331	209	2.67	57.1	4	12455	0.00716	97	601	24	123790	0.00561	124	16	12
Mean		0.00307	229	2.27	58.6	5.5	14806	0.00596	119	641	30.4	158042	0.00499	159	15.3	27.3
7	2800	0.00299	232	3.61	56.6	6	12881	0.00802	87	470	24	106316	0.00224	310	12	24
8		0.00289	240	2.23	87.7	6	20871	0.00476	146	854	24	210632	0.00324	214	18	12
1		0.00390	178	2.97	91.4	6	15635	0.00761	91	682	24	138053	0.00430	161	17	16
3		0.00288	241	2.58	59.3	12	18018	0.00445	156	672	24	174794	0.00404	171	16	24
Mean		0.00316	223	2.85	73.7	7.5	16851	0.00621	120	669	24.0	157449	0.00345	214	15.8	19.0
6	3600	0.00237	292	2.36	81.7	6	25353	0.00475	146	987	49	293607	0.00419	166	22	16
2	3600	0.00210	329	2.15	86.3	8	27818	0.00439	158	1011	24	290871	0.00430	161	24	16
7	3600*	0.00261	265	2.48	80.3		24131	0.00491	141	1007		274858	0.00396	175	19	23
8	3600*	0.00259	268	2.05	134.7		29116	0.00431	161	1134		262505	0.00421	165	26	23
Mean		0.00242	289	2.26	95.8		26604	0.00456	151	1035		280460	0.00416	167	22.8	19.5

*Split dose

Table 4.3 Adverse Experiences

	Dose (mg)	200	800	1200		2000				3600		
	Subject	2	8	5	6	2	4	6	9	6	2	7
	Time*											
Headache	4	1			1							
	6	1		1					2		1	
	8	1		1					2		1	
	12			1		2-3			1		2	
	16			1		2-3					1	2-3
	24										1	
	48						1					
	72		1									
Nausea	2				2							
	6										1	
	12			2								
Abdominal discomfort	8										2	
	10									1		
	12										1	
Vomiting	12			2								
Aching in arms	48			1 1								
Tired after mild exertion	48									1		
	72									1		

* Time of symptom onset in hours

Key : 1 = mild; 2 = moderate; 3 = severe intensity

FIGURES

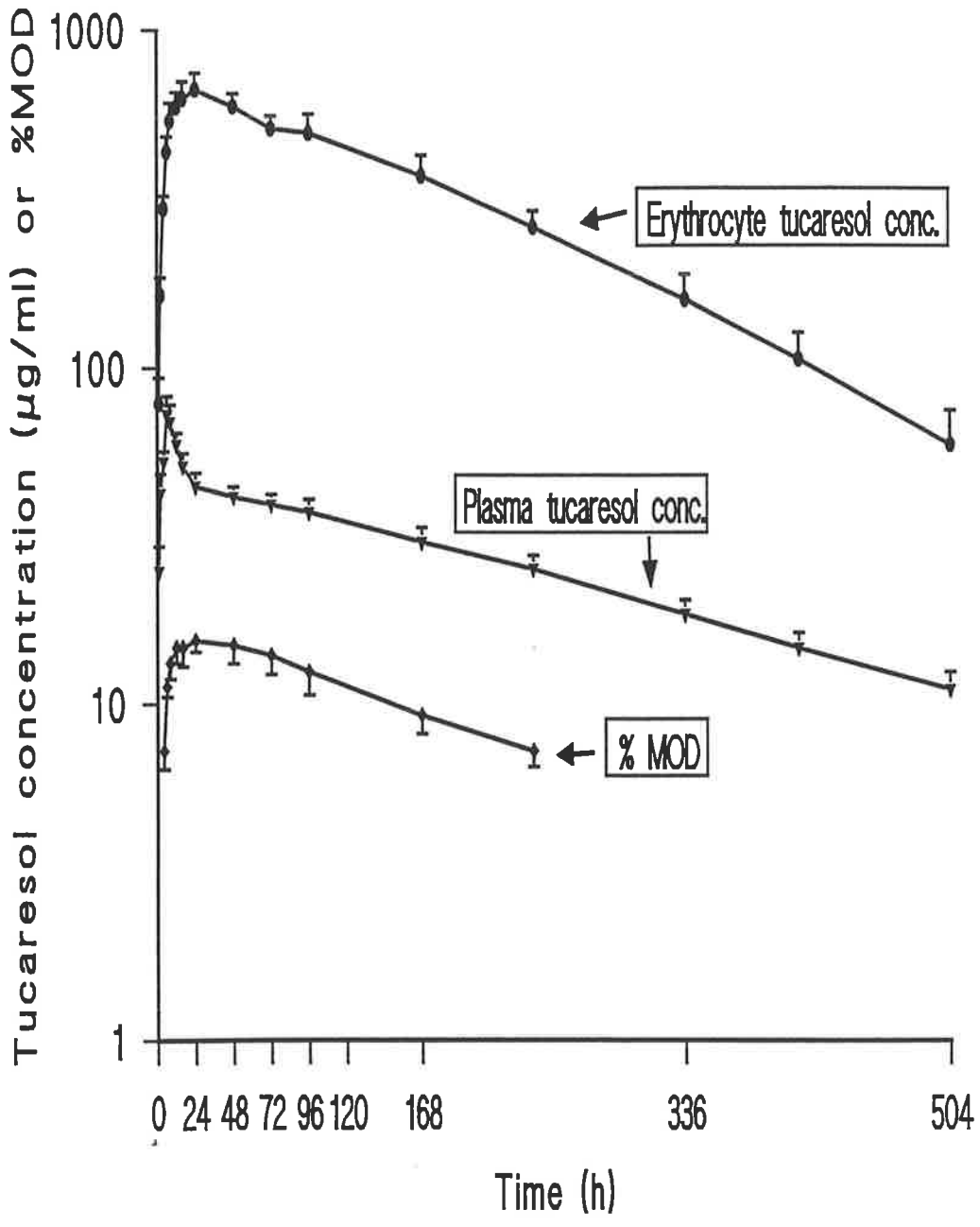


Figure 4.1 Mean concentration-time profiles for tucaresol in erythrocytes and plasma and %MOD-time curve following 2800 mg tucaresol.

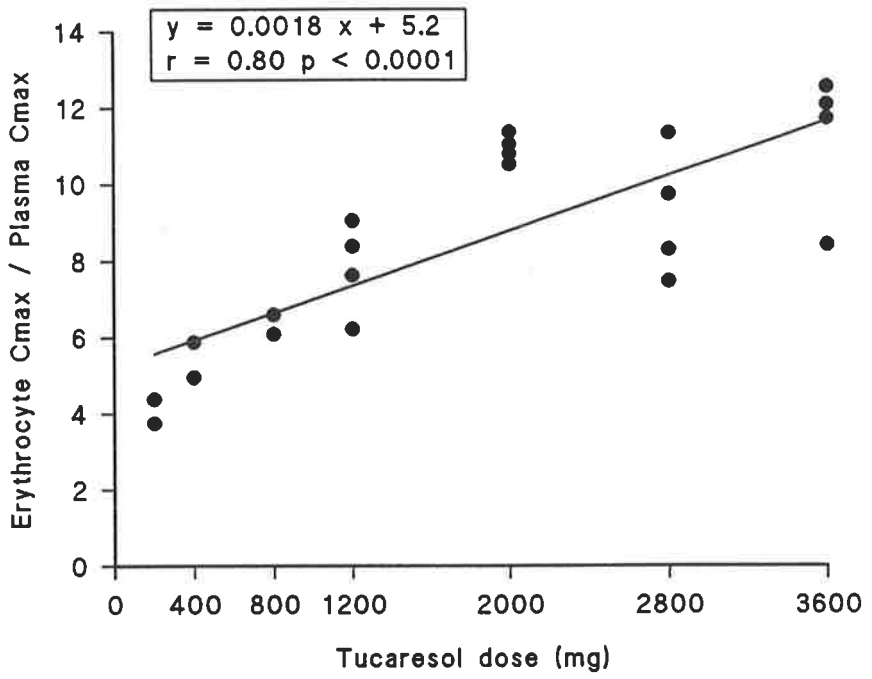


Figure 4.2 Erythrocyte C_{max} / plasma C_{max} ratio as a function of tucaresol dose.

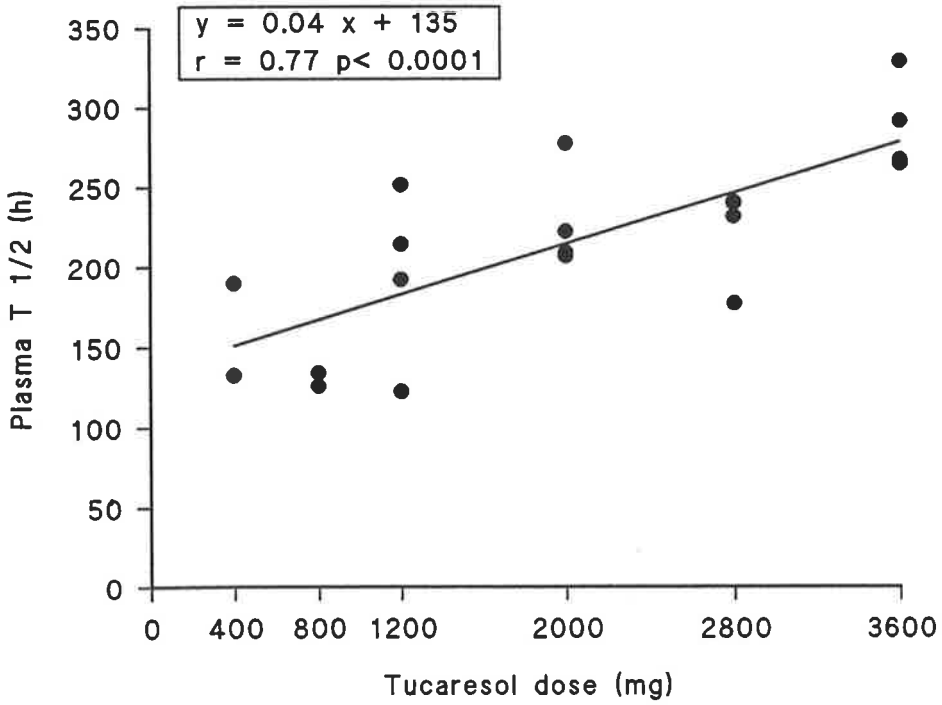


Figure 4.3 Relationship between tucaresol dose and plasma half-life.

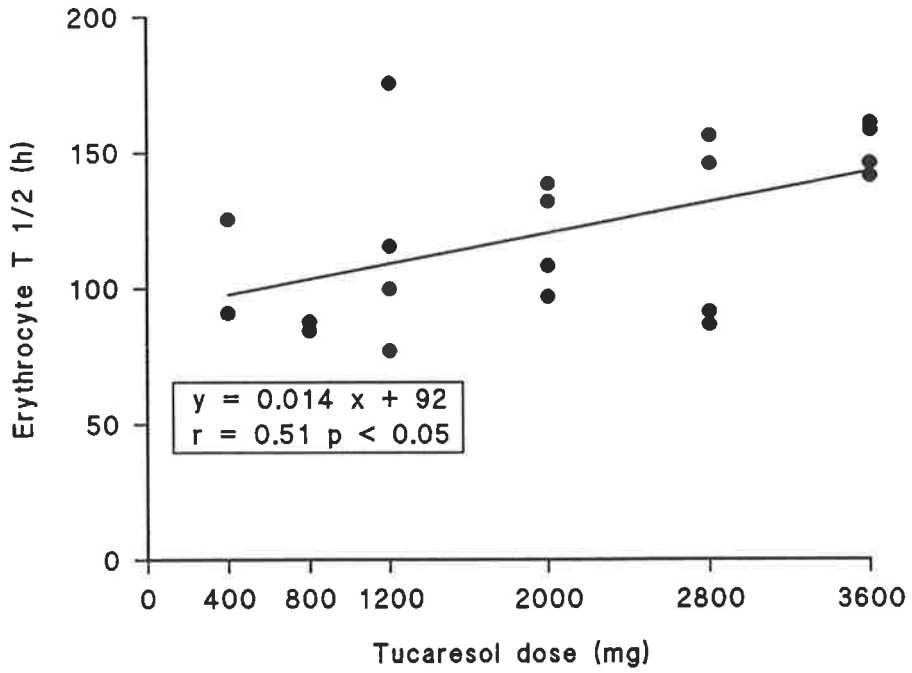


Figure 4.4 Relationship between tucaresol dose and erythrocyte tucaresol half-life.

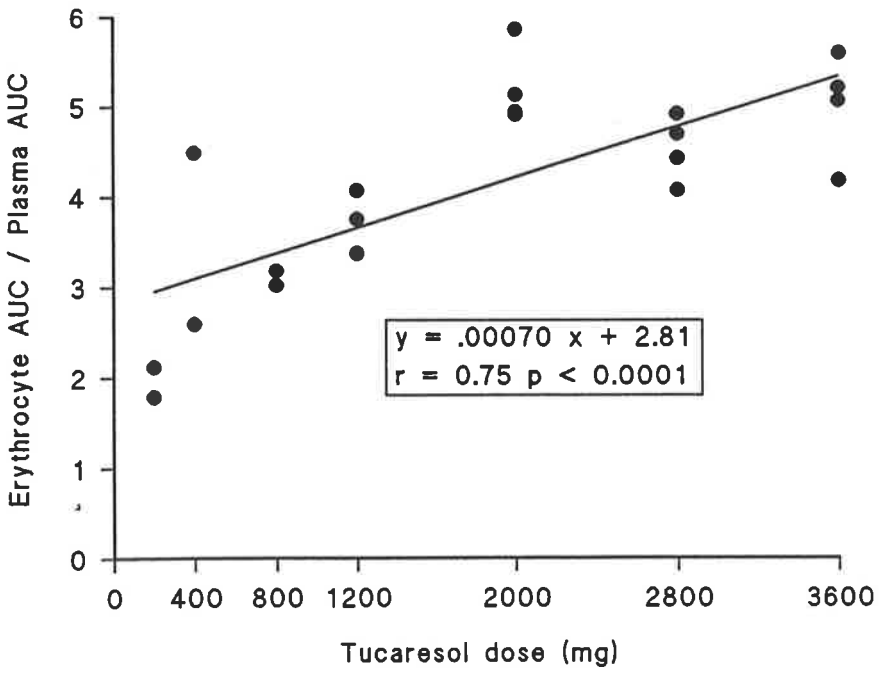


Figure 4.5 Erythrocyte / plasma AUC ratio for tucaresol as a function of tucaresol dose.

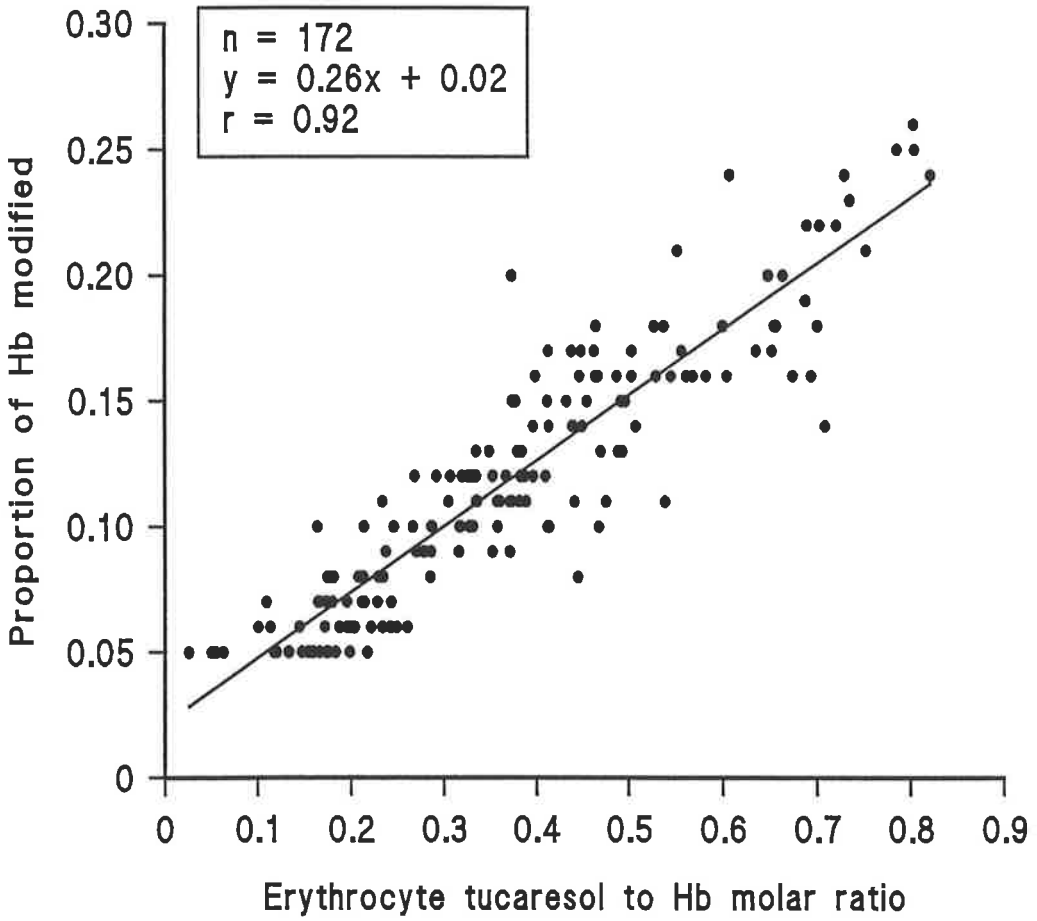


Figure 4.6 Proportion of Hb modified (%MOD / 100) as a function of erythrocyte tucaresol to haemoglobin (Hb) molar ratio.

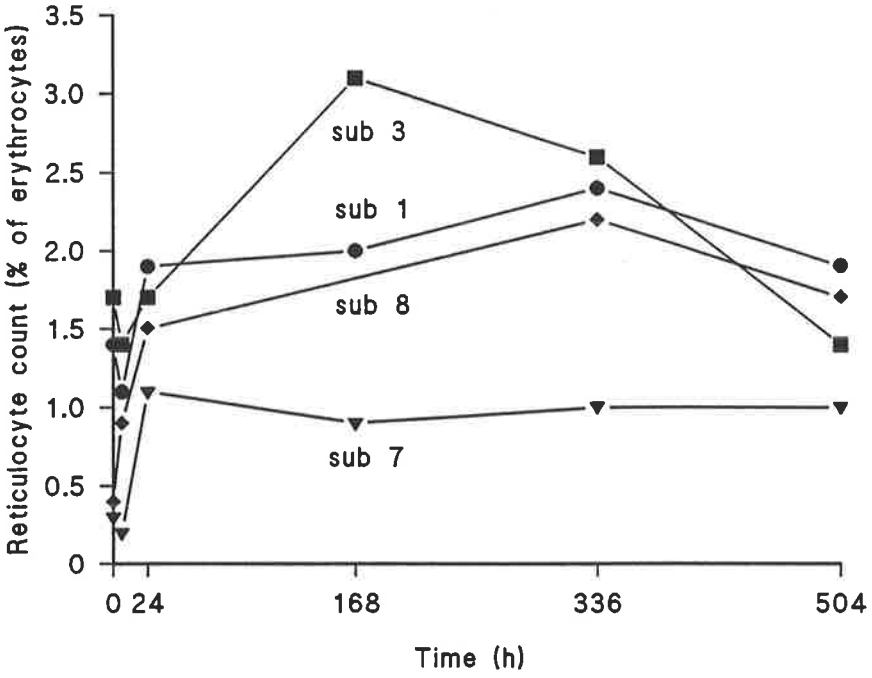


Figure 4.7 Reticulocyte counts for individual subjects following 2800 mg tucaresol.

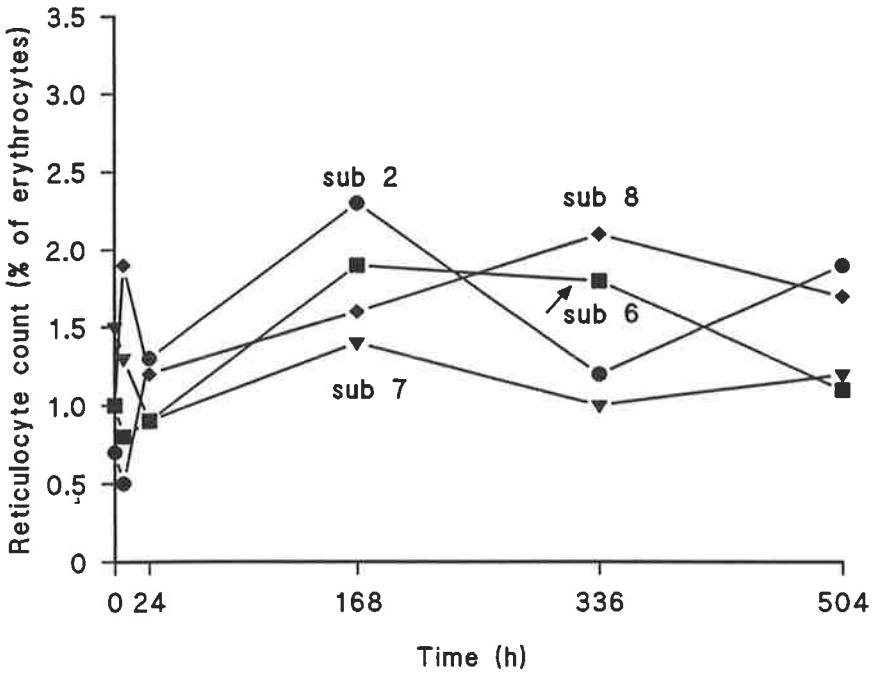


Figure 4.8 Reticulocyte counts for individual subjects following 3600 mg tucaresol.

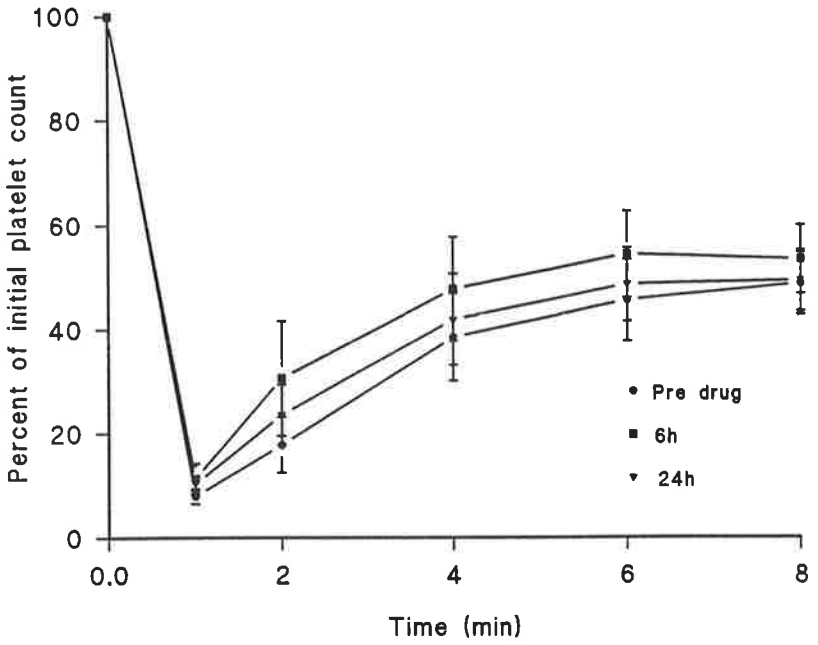


Figure 4.9 Mean platelet counts in whole blood as percent of initial count following addition of 0.5 μg/ml collagen *ex vivo* for 4 subjects before and at 6 and 24 h after 2800 mg tucaresol.

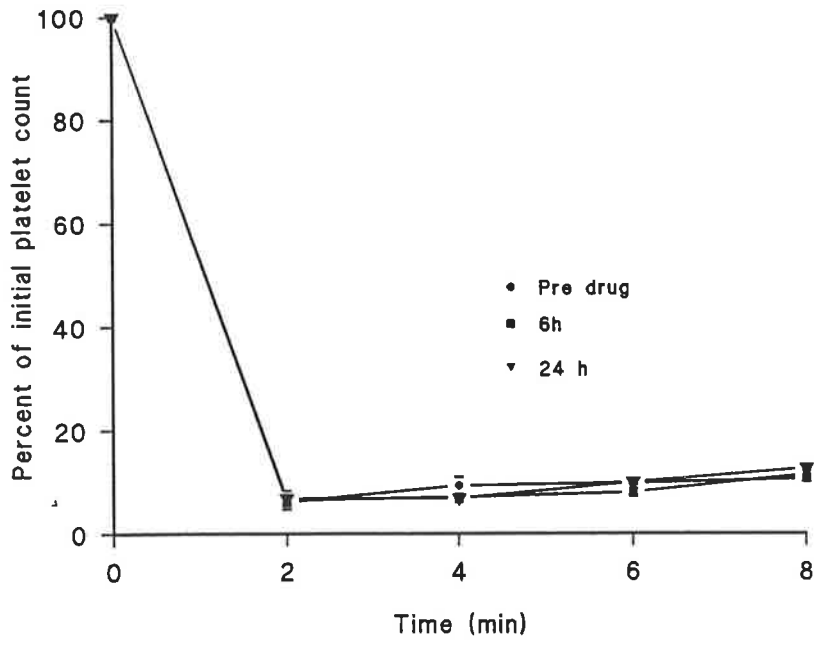


Figure 4.10 Mean platelet counts in whole blood as percent of initial count following addition of 2 μM collagen *ex vivo* for 4 subjects before and at 6 and 24 h after 2800 mg tucaresol.

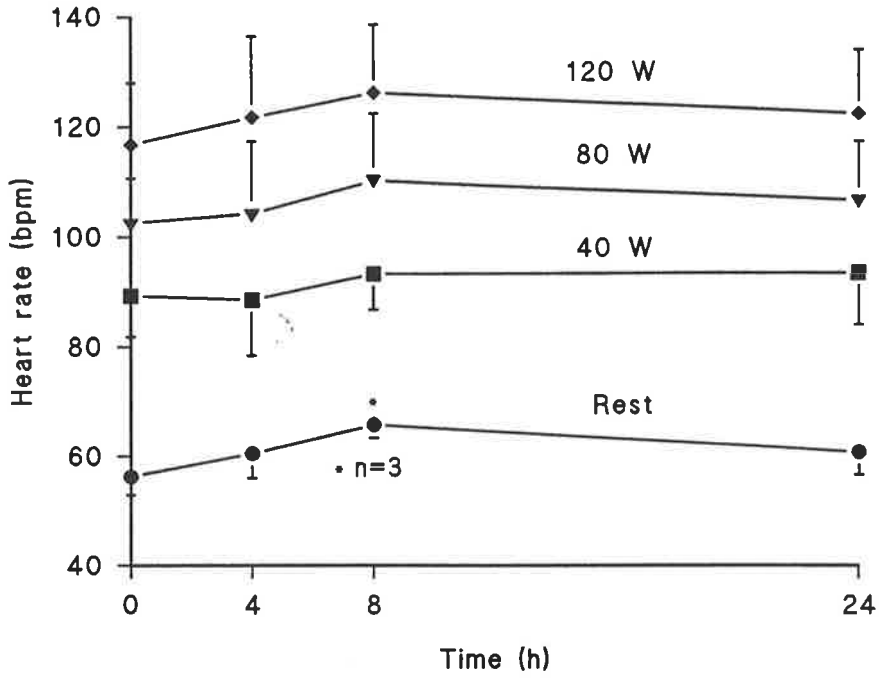


Figure 4.11 mean heart rates at rest and at 40, 80 and 120 W exercise after 2800 mg tucaresol.

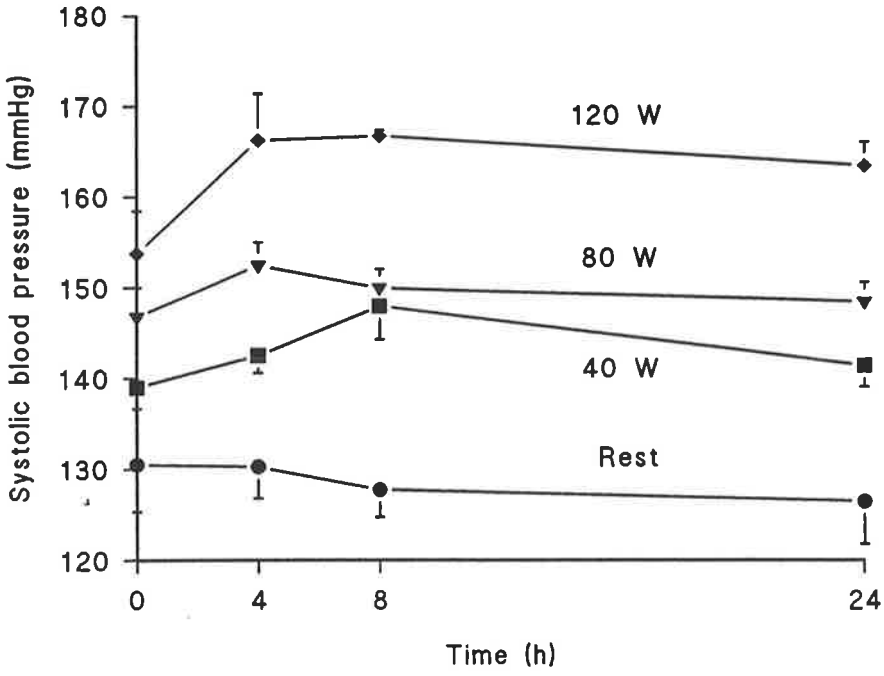


Figure 4.12 Mean supine blood pressure at rest and at 40, 80 and 120 W exercise 2800 mg tucaresol.

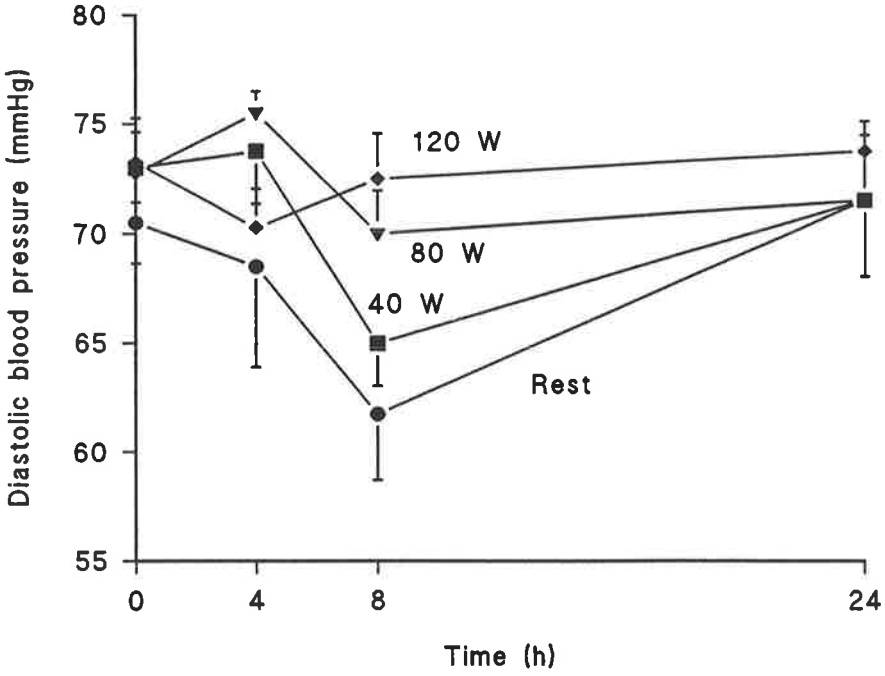


Figure 4.13 Mean diastolic blood pressure at rest and at 40, 80 and 120 W exercise following 2800 mg tucaresol.

CHAPTER 5

THE EFFECTS OF A TITRATED LOADING DOSE OF TUCARESOL IN HEALTHY MALE VOLUNTEERS

5.1 Introduction and Rationale

The previous study was terminated before the designated study end point of 40%MOD was achieved because the half life of tucaresol in man was longer than expected and consequently the single dose escalating design (allowing for "washout") would have required the participation of the volunteers for a period of months. As the %MOD reached in that study did not span the anticipated therapeutic range, this study investigated the effects of higher %MOD levels using a placebo-controlled study design to more extensively evaluate the tolerability and pharmacodynamics of the compound.

5.2 Objectives

The objectives of this study were :

1. To investigate the effects of multiple oral doses of tucaresol resulting in approximately 15%, 25% and 32.5%MOD of haemoglobin in healthy volunteers on:
    - adverse experiences
    - the cardiovascular response to graded moderate exercise
    - psychometric test performance.
2. To investigate the pharmacokinetics of tucaresol after multiple dosing.
3. To determine whether target %MOD levels can be achieved reliably using individually titrated doses.

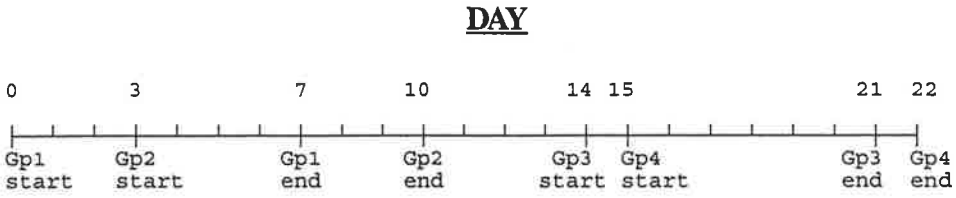
5.3 Study design

5.3.1 General

Twelve volunteers took part in a double blind, randomised, placebo-controlled study design consisting of two unequal parallel groups receiving tucaresol (n = 8) or placebo (n = 4). The volunteers were resident at the Wellcome Clinical Investigation Unit (WCIU) for 7 days so that drug administration could be carried out under medical supervision and volunteers were followed up on an ambulatory basis at their place of work for a further 34 days. The study randomisation code was then broken so that only those volunteers who received tucaresol underwent additional blood sampling for a further 35 days to characterise the terminal elimination phase of tucaresol.

The study design involved the oral administration of three doses of tucaresol at 48-hour intervals to achieve approximately 15, 25 and 32.5%MOD. The effects of this %MOD on the response to graded moderate exercise and on psychometric performance were assessed on days 2, 4 and 6 of the study. Haematology and biochemistry were monitored throughout the study.

Four groups of three volunteers entered the study, each comprising two volunteers taking tucaresol and one taking placebo. The volunteer entry timetable (see below) allowed the initial assessment of the progress of the first group before committing the second group of three volunteers to exposure to tucaresol. As the first two groups completed the residential phase of the study without adverse experiences which might have contra-indicated the further dosing of volunteers, two further groups of three volunteers (two active drug and one placebo in each) were entered into the study separated by one day.



5.3.2 Design considerations

The advantages of giving the dose of tucaresol in three fractions included the avoidance of the gastrointestinal adverse experiences detected after a single large dose of tucaresol in the previous study and the opportunity to assess any adverse experiences before continuing to a further dose of tucaresol. Due to the dose proportionality of C_{max} within each volunteer in the previous study and the close correlation between tucaresol whole blood concentration at 24 hours and %MOD, it was possible to use the %MOD at 24 hours after the first and second doses to titrate the individual volunteer to the target %MOD.

5.3.3 Statistical considerations

The number of volunteers selected for this study and the balance between active and placebo groups was chosen to reflect both the need to acquire as much information as possible about any adverse experiences associated with tucaresol and the reliable detection of any effects of tucaresol in the exercise and psychometric tests.

A study investigating the effects of changes in %MOD (Nicholls *et al*, 1989a) with valeresol detected significant effects of %MOD on exercise with six volunteers and published studies have reported significant effects of reduced tissue oxygen delivery on selected psychometric tasks with a similar sample size to that proposed for this

study (Schaffler *et al*, 1981 and 1988), although these studies were of a crossover design.

5.3.4 Exercise tests

As stated in Chapter 4, the increased oxygen affinity of haemoglobin caused by to tucaresol could theoretically reduce the tissue oxygen delivery per unit volume of blood. To maintain tissue oxygen delivery, compensatory mechanisms such as increased tissue blood flow by vasodilation with increased cardiac output might occur. Evidence of the vasodilator effects of tucaresol has been detected in the anaesthetized dog after the intravenous infusion of tucaresol to 44%MOD. In a previous study (Nicholls *et al*, 1989a) using valeresol, a greater effect of the drug was observed on the augmentation of exercise induced heart rate than on resting heart rate.

This study investigated the effects of tucaresol and placebo on heart rate and cardiac output at rest and in response to moderate graded exercise at 50, 85 and 120 W. As the relative contributions of stroke volume and heart rate to increasing cardiac output vary with posture (Leyk *et al*, 1994), the exercise test was carried out in both the supine and seated positions. These studies were designed to determine whether there is a cardiovascular effect of tucaresol which may be relevant to sufferers of sickle cell disease, in whom exercise is already known to pose a risk (Martin *et al*, 1989). A Borg rating scale test (Borg, 1970), designed to determine the perceived difficulty of the required work rate, was undertaken during the last 30 seconds of each exercise period.

5.3.5 Psychometric tests

Psychometric tests were included in the study design because of the possibility that psychometric performance might be affected by the possible reduced tissue oxygen delivery per unit volume of blood. Examples of both cognitive and motor tasks were included.

A mood rating scale was included as sickle cell disease sufferers may experience an improvement in their quality of life following treatment with tucaresol. It was important to determine whether this could be due to an amelioration of the disease state or to a specific mood enhancing effect of tucaresol.

5.3.6 Safety considerations

Vital signs (supine blood pressure, heart rate and respiration rate) in resting volunteers were recorded pre-dosing and on days 0-6 of the study and at various subsequent times. Volunteers' ECG status was monitored at all times in the Investigation Unit using an ECG telemetry system.

5.4 Subjects, Protocol and Methods

5.4.1 Subjects

The inclusion and exclusion criteria for this study were the same as for the previous study. Subjects gave written informed consent after a full oral and written explanation of the study, and were paid an honorarium on completion.

5.4.2 Protocol

The study protocol was approved by the Wellcome Protocol Review Committee and the Camberwell Health Authority Ethics Committee.

5.4.3 Study drug

Tucaresol tablets of 200 and 400mg and matching placebo were supplied by The Wellcome Foundation Ltd, Dartford, Kent.

5.4.4 Drug administration and dosages

5.4.4.1 Selection of tucaresol dose

The results of the previous study suggested that the %MOD produced by a given dose of tucaresol was dependent to some extent upon body weight. Simple regression analysis revealed that the best estimate of the dose required for a given %MOD could be obtained from the relationship expressed in Equation 5.1:

$$DOSE\ (mg) = \frac{REQUIRED\ \%MOD \times WEIGHT\ (kg)}{0.434} + 1.01 \quad \textbf{Eqn 5.1}$$

This equation was used to calculate the doses predicted to achieve 15%MOD in volunteers in the weight range 50 - 100 kg. The doses were rounded to the nearest 200 mg as this was the smallest unit dose available. These represent the initial doses used on day 1. The range of the calculated expected %MOD after the initial dose was 14.4% - 15.7% (because of dose rounding).

Twenty-four hours after the first dose (08.00 hours : day 2), a blood sample was taken for haemoglobin modification determination in duplicate. The arithmetic mean of the two %MOD determinations was used to calculate the second dose employing Equation 5.2 (see below). The calculation was performed by a pharmacist and was checked by the scientist measuring the %MOD levels. These personnel were not otherwise involved in the clinical phase of the study in order to maintain blinding.

X1 = oral dose of tucaresol predicted to produce 15%MOD at 24 hours

X2 = oral dose of tucaresol predicted to produce 25%MOD 24 hours later

%MOD1 = %MOD actually produced by X1 at 24 hours

%MOD2 = %MOD actually produced by X2 at 72 hours

Assuming dose proportionality between dose, C_{max} and %MOD, and ignoring drug elimination:

$$\frac{25 - \%MOD1}{X2} = \frac{\%MOD1}{X1}$$

However, X2 must incorporate a "top-up" dose to allow for the amount of X, eliminated between 24 and 72 hours. This was estimated to be:

$$\frac{19.1 \cdot X1}{100}$$

based on the percentage difference between the erythrocyte concentration of tucaresol at 24 hours and 72 hours following 2000 mg, 2800 mg and 3600 mg (single dose) from the previous study.

Therefore:

$$X2 = \left(\frac{25 - \%MOD1 \;.\; X1}{\%MOD1}\right) + \left(\frac{19.1 \;.\; X1}{100}\right) \qquad \textbf{Eqn 5.2}$$

Similarly, the third dose was estimated from the mean %MOD of a sample taken 24 hours after the second dose (08.00 hours : day 4) using Equation 3.

$$\frac{32.5 - \%MOD2}{X3} = \frac{\%MOD2}{X2 + \left(1 - \frac{19.1}{100}\right) . X1}$$

Therefore:

$$X3 = \left(\frac{32.5 - \%MOD2}{\%MOD2}\right) . \left(X2 + \left(1 - \frac{19.1}{100}\right) . X1\right) . \frac{19.1}{100} . X2 + \frac{13.4}{100} . X1$$

Eqn 5.3

Where X3 is the oral dose of tucaresol predicted to produce 32.5%MOD 24 hours later, and where 13.4 is the percentage of X1 eliminated between 72 hours and 120 hours after dosing (based on an adjustment [assuming constant rate of elimination] of the percentage difference between the erythrocyte concentrations of tucaresol at 72 hours and 168 hours following 2000 mg, 2800 mg and 3600 mg (single dose)).

The second and third doses were also rounded to the nearest 200 mg unit dose. All doses for all subjects based on the above calculations were dispensed and checked by the same personnel (pharmacist and scientist) before placing into sealed pots for transfer to the Investigation Unit. The maximum number of 200 mg tablets administered in any dose was 1. To maintain blinding, similar adjustments were also made to the numbers of tablets for the volunteers receiving placebo.

5.4.4.2 Administration

The dose of tucaresol was administered as a combination of 400 mg and 200 mg tablets with 250 ml of water.

5.4.5 Schedule

Volunteers were required to abstain from alcohol for 7 days before the start of the study. They were taken to the Investigation Unit at King's College Hospital by taxi on the morning of day 0. Volunteers practised the psychometric and exercise tests until no further performance improvement was apparent (to plateau) and then produced baseline (pre-drug) measurements.

There were no restrictions on food or fluids on day 0. Volunteers fasted from food from 00.00 hours on days 1, 3 and 5 (i.e. dosing days). Beverages alone were allowed between 10.00 hours and 12.00 hours on days 1, 3 and 5 and lunch was provided at the time stipulated in the schedule. Breakfast was provided on non-dosing study days.

The volunteers were resident for 7 days after which they were followed up on an ambulatory basis at their place of work for a further 34 days, at which time the study randomisation code was broken. Those volunteers who had received tucaresol were followed up for a further 35 days. This follow up consisted of 4 blood samples for tucaresol determination and full blood counts on days 48, 55, 62, 69 and 1 sample for tucaresol determination, full blood count and clinical biochemistry on day 76.

5.4.5.1 Blood samples

The whole blood haemolysate and plasma levels of tucaresol and the %MOD were determined on days 0 - 7 inclusive and on days 9, 11, 13, 17, 20, 27, 34 and 41 of the study. Tucaresol determinations were continued as above for those volunteers on active treatment.

Full blood counts and biochemistry were measured on days 1, 3, 5, 7, 9, 11, 13, 20, 27, 34 and 41 for safety monitoring and haematocrit measurement and continued as above for those volunteers on active treatment. Blood coagulability was determined pre-dose, at peak %MOD (day 6) and on day 41.

5.4.5.2 Urine samples

Urine was collected over 24 hours on days 1 - 7 inclusive. Twenty ml spot urine samples were collected on days 9, 11, 13, 17, 20, 27, 34 and 41.

5.4.6 Laboratory Methods

Assays of tucaresol in plasma and haemolysate, plasma biochemistry, full blood counts and coagulation and determination of %MOD were performed as described in the previous chapter.

5.4.7 Clinical measurements and procedures

5.4.7.1 Exercise tests

The cardiovascular response to moderate graded exercise was investigated in two body positions. 'Supine' exercise took place on a cycle ergometer (Lode) lying

inclined at 7.5° to the horizontal at rest (0 W) and at incrementing work rates of 50, 85 and 120 W for 3 minutes at each level. 'Seated' exercise took place on the same ergometer in the conventional seated cycling position at rest (0 W) and at work rates of 50, 85 and 120 W for 3 minutes at each level. At some point during the final 30 seconds of each exercise stage a Borg rating scale was recorded. Exercise was suspended for 1 minute between ascending work rates to allow blood pressure, heart rate and respiration rate to be monitored (Hewlett Packard HP78352A automatic sphygmomanometer). Heart rate before exercise and at the end of each 3 minute period of exercise was measured from a 10 second portion of the monitor rhythm strip.

Cardiac output and stroke volume were measured by impedance plethysmography using a BOMED NCCOM3 analyser (Bomed Medical Manufacturing, Irvine, California, USA) which allowed the monitoring of a number of cardiovascular parameters of individual heart beats including stroke volume and cardiac output (Bernstein, 1986a). The commercially available NCCOM3 monitor continuously displays cardiac output and other cardiovascular parameters derived from the thoracic bioimpedance signal. The device applies a small alternating current across the thorax, and records the applied signal through separate electrodes. The magnitude and waveform of the rate of change of impedance signal (dZ/dt) is correlated to aortic blood flow and varies with the cardiac cycle. Volume and velocity of blood within the aorta both contribute to the changes in the bioimpedance signal. Stroke volume is calculated using the Sramek equation (Bernstein, 1986b) from the magnitude and timing of these changes. The heart rate

is derived from the ECG signal. The data from approximately 10 consecutive heart beats were transmitted to an IBM-compatible personal computer using a communications software program (Procomm Plus).

5.4.7.2 Borg scale

This test provided a measure of the perceived exertion required for a physical task by means of a rating scale (Borg, 1970). The scale consisted of 15 grades from 6-20 giving rating of perceived exertion (RPE). The subject was asked to rate the degree of exertion as accurately as possible by either pointing at or naming a number from the scale below.

6		14	
7	Very, very light	15	Hard
8		16	
9	Very light	17	Very hard
10		18	
11	Fairly light	19	Very, very hard
12		20	
13	Somewhat hard		

RPE has been shown to correlate well with heart rate after exercise (Borg, 1970).

5.4.7.3 Psychometric tests

Choice reaction time, the Stroop test, a mood adjective checklist and a visual analogue mood rating scale were assessed according to the study schedule using an

IBM-compatible personal computer equipped with a touch sensitive screen. The task battery took approximately 13 minutes to complete.

5.4.7.3.1 Choice reaction time

This task has been designed to provide a combination measure of cognitive and motor processes and has been shown to be sensitive to a variety of drugs and physical stresses in man (Wesnes *et al*, 1987).

The task required subjects to make a rapid response to an unpredictable sequence of signals and consisted of an arc of five display points and a "home" point. The volunteer was obliged to place his finger on the "home" point until one of the display points turned red. The volunteer was then required to touch the display point as quickly as he could and then to return his finger to the "home" point. The next display point was not illuminated until a response was registered by a return to the "home" point. The display points illuminated in a random order and at a pseudo-random (random within limits) time interval to prevent anticipation. The dependent variable was reaction time in milliseconds.

5.4.7.3.2 Mood adjective checklist

The Mood Adjective Checklist (MACL) consists of 48 adjectives describing a range of mood states. Subjects were required to judge the degree to which each adjective applied to their mood using a four-point scale: 'definitely', 'slightly', 'slightly not', and 'definitely not'. The dependent variables were dimensionless rating scores.

5.4.7.3.3 Stroop test

The Stroop test explores the conflict between two competing sources of information and the cognitive processing required to resolve that conflict. The volunteer was presented with a word spelling a colour name printed in a different colour (e.g RED printed in blue). The volunteer was asked to respond with the colour in which the word was printed (and not the colour that the word spelt) from a menu of colour blocks. The subjugation of the natural response to answer with the named colour is a measure of high level cognitive processing (Stroop, 1935). The dependent variables were the total response time in seconds and the number of correct responses.

5.4.7.3.4 Visual analogue mood rating scales

The volunteer was asked to rate his mood on a number of 100 mm lines anchored by pairs of semantic opposites (e.g. relaxed and tense, happy and unhappy) (Bond and Lader, 1974). The dependent variable was distance from the appropriate end of the line in millimetres.

5.4.8 Data analysis

5.4.8.1 Pharmacokinetic analysis

Tucaresol plasma concentration data generated by the study were analysed using functions and algorithms in Microsoft EXCEL and QuickBASIC using the same noncompartmental approach and equations described in the previous chapter. Nominal doses were adjusted for potency as listed on the Certificates of Analysis to give exact doses which were used for all calculations. For all the pharmacokinetic

calculations, zero time was taken as the time of the morning dose of the first dose. For the last 6 subjects, this was in fact the second half of the first dose with the first half given at midnight of the previous day (see Section 5.5.2). The zero time plasma concentration was also taken as zero for these subjects, although a non-zero value was measured.

5.4.8.2 Statistical analysis

5.4.8.2.1 Exercise tests

Comparisons were made between treatments (tucaresol/placebo) for systolic blood pressure, diastolic blood pressure, heart rate, stroke volume, cardiac output, respiration rate and the Borg Rating scale using analysis of variance. For each variable, change from baseline (day 0) was subjected to analysis of variance. The analysis took into account sources of variation due to treatments. Comparisons were made for measurements taken following both seated and supine exercise at each assessment time (days 2, 4 and 6) and at each work rate (0, 50, 85 and 120 W). 95% confidence intervals for the differences between treatments were calculated.

5.4.8.2.2 Psychometric tests

For each psychometric test, a comparison was made between treatments (tucaresol/placebo) for each variable of interest at each assessment time (days 2, 4 and 6) using analysis of variance. For each variable, change from baseline (day 0) was subjected to analysis of variance. The analysis took into account sources of

variation due to treatments. 95% confidence intervals for the differences between treatments were calculated.

5.5 **Results**

5.5.1 Subjects

Details of subjects are in Table 5.1. Their ages and weights ranged between 20-40 years and 61-98 kg.

5.5.2 Protocol compliance and modifications

Due to minor gastrointestinal symptoms after the first two doses in the first cohort of volunteers (see section 5.5.7, adverse experiences), the last dose for the first cohort, the last two doses for the second cohort and all doses for volunteers in subsequent cohorts were split into two fractions of approximately equal size, administered 8 hours apart. The first fraction was administered at 00.00 hours on the scheduled day of dosing and the second fraction was administered at the scheduled time (approximately 08.00) on the scheduled day of dosing (day 1, 3 or 5). A summary of the dosing history is in Table 5.2.

Due to adverse experiences that were thought possibly to have been due to viral infection (see section 5.5.7, adverse experiences), viral serology was performed retrospectively from stored plasma from the screening medical examination and from stored plasma taken during the follow up phase of the study.

5.5.3 Doses administered

A summary of individual doses is presented in Table 5.2. Mean (range) total dose administered was 6075 mg (5200-7000).

5.5.4 Pharmacokinetics

Mean log concentration-time profiles for tucaresol in plasma and erythrocytes are plotted in Figure 5.1. Inspection of the individual curves showed them to be triphasic, with the second phase being faster than either the first or third. Calculated model-independent pharmacokinetic parameters for plasma and erythrocytes are given in Table 5.3. Mean (SD) maximum concentrations of tucaresol in plasma and erythrocytes occurred on day 5 or 6 and were 81.4 ± 4.1 and 1459 ± 162 $\mu g\ ml^{-1}$ respectively. The elimination rate constant k_e was calculated from the middle portion of the elimination phase as it represented the majority of the curve. Mean (SD) half-life was 257 ± 39 hours from plasma and 157 ± 29 hours from erythrocytes. Mean (SD) apparent plasma clearance was 2.77 (0.37) ml/min and apparent volume of distribution was 60.9 (8.6) l.

5.5.5 Haemoglobin modification - %MOD

The individual and mean maximum %MOD, t_{max} and half-life values are also given in Table 5.3 and a summary of the %MOD measurements is given in Table 5.4. The mean %MOD-time profile is also plotted on semilogarithmic axes in Figure 5.1. Mean (SD) %MOD on days 2, 4 and 6 were 17.8 (1.51), 28.0 (2.61) and 33.1 (2.80) compared with the target levels of 15, 25 and 32.5% respectively. Target and achieved %MOD values are plotted in Figure 5.2. Haemoglobin modification was

not detected after day 28. Figure 5.3 shows the relationship between the proportion of Hb modified (%MOD x 100) and the erythrocyte tucaresol to Hb molar ratio.

5.5.6 Cardiovascular and psychometric data

All differences between treatment refer to the difference tucaresol minus placebo.

5.5.6.1 Vital signs (BP, HR)

There were no consistent trends for differences between treatment in resting heart rate or blood pressure.

5.5.6.2 Exercise tests

5.5.6.2.1 Systolic Blood Pressure - Seated Exercise

Table 5.5 contains the mean change from baseline in systolic blood pressure following seated exercise on days 2, 4 and 6. Table 5.6 contains the point estimates and 95% CI's for the differences between treatments. The mean change from baseline following tucaresol and placebo revealed a consistently greater change from baseline on day 6 compared to day 2 at each exercise level (Figure 5.4). The 95% confidence intervals showed no statistically significant differences between treatments on days 2, 4 or 6.

5.5.6.2.2 Systolic Blood Pressure - Supine Exercise

Table 5.7 contains the mean change from baseline in systolic blood pressure following supine exercise on days 2, 4 and 6. Table 5.8 contains the point estimates and 95% CI's for the differences between treatments. The mean change

from baseline following tucaresol showed an increase in systolic blood pressure at day 2 at all exercise levels but by day 6 systolic blood pressure had fallen at all exercise levels except 120 W (Figure 5.5). The mean changes from baseline following supine exercise were more varied than those following seated exercise for the tucaresol treatment group. Generally, smaller mean differences from baseline were recorded following supine exercise compared to seated exercise. The 95% confidence intervals at day 2 and day 4 indicated a statistically significant difference in change in systolic blood pressure between treatments of 0-27 and 4-30 mmHg respectively at the 120 W exercise level. The 95% confidence interval at day 2 at the 85 W exercise level also showed a statistically significant difference (2-31 mmHg).

5.5.6.2.2 Diastolic Blood Pressure - Seated Exercise

Table 5.9 contains the mean change from baseline in diastolic blood pressure following seated exercise on days 2, 4 and 6. Table 5.10 contains the point estimates and 95% CI's for the differences between treatments. By day 4 the mean change from baseline following tucaresol showed decreases from baseline in diastolic blood pressure at each exercise level. The mean changes were more varied following placebo. The 95% confidence intervals showed a statistically significant difference between treatments on day 2 at the 50 W exercise level (-17-0) and on day 6 at the 120 W exercise level (-16 to -2 mmHg) (Figure 5.6). This former result may be spurious as a significant treatment difference was not observed at days 4 and 6 at the 50 W exercise level.

5.5.6.2.3 Diastolic Blood Pressure - Supine Exercise

Table 5.11 contains the mean change from baseline in diastolic blood pressure following supine exercise on days 2, 4 and 6. Table 5.12 contains the point estimates and 95% CI's for the differences between treatments. By day 4, the mean changes from baseline following tucaresol at all exercise levels except 0 W showed mean decreases from baseline in diastolic blood pressure. The mean changes following placebo showed decreases at all exercise levels on days 4 and 6 (Figure 5.7). The mean changes from baseline following supine exercise were generally greater following tucaresol whereas the changes from baseline following seated exercise were generally greater following placebo. The 95% confidence intervals revealed no statistically significant differences between treatments.

5.5.6.2.4 Heart Rate - Seated Exercise

Table 5.13 contains the mean change from baseline in heart rate following seated exercise on days 2, 4 and 6. Table 5.14 contains the point estimates and 95% CI's for the differences between treatments. The mean changes from baseline in heart rate for the tucaresol treatment group showed a steady increase from day 2 to day 6. The mean changes following placebo at all exercise levels and all days except day 2 at the 0 W exercise level showed increases in heart rate (Figure 5.8). The 95% confidence intervals showed for days 4 and 6 at the 120 W exercise level (3-36 and 8-38 bpm) and at day 6 at the 85 W exercise level (3-41 bpm) a statistically significant difference between treatments.

5.5.6.2.5 Heart Rate - Supine Exercise

Table 5.15 contains the mean change from baseline in heart rate following supine exercise on days 2, 4 and 6. Table 5.16 contains the point estimates and 95% CI's for the differences between treatments. The mean changes for the tucaresol treatment group were highest at the 120 W exercise level for each day. At all exercise levels and all days increases were seen in heart rate for the tucaresol treatment group (Figure 5.9). The means following placebo were variable. The mean changes from baseline following both seated and supine exercise were generally greater following tucaresol compared to placebo. The 95% confidence intervals showed a statistically significant difference between treatments at 120 W on all days (9-22, 11-27 and 13-37 for days 2, 4 and 6). Significant differences were also seen at 50 W on days 4 and 6 (3-20 and 0-33 respectively) and at 85 W on day 6 (8-24).

5.5.6.2.6 Respiration Rate - Seated and Supine Exercise

There were no trends for changes between treatments in respiration rate during either seated or supine exercise.

5.5.6.2.7 Borg Rating Scale - Seated and Supine Exercise

There were no trends for changes between treatments in the Borg Rating scale during either seated or supine exercise.

5.5.6.2.8 Stroke Volume

Table 5.17 contains the mean change from baseline in stroke volume following seated exercise on days 2, 4 and 6. Table 5.18 contains the point estimates and

95% CI's for the differences between treatments. A mean decrease in stroke volume from baseline was seen at all exercise levels on each day following tucaresol (Figure 5.10). Following placebo the mean changes were more variable with many increases. The 95% confidence intervals showed no statistically significant differences between treatments.

Table 5.19 contains the mean change from baseline in stroke volume following supine exercise on days 2, 4 and 6. Table 5.20 contains the point estimates and 95% CI's for the differences between treatments. Following tucaresol a mean decrease in cardiac stroke volume was seen at each exercise level and at each day, the mean decreases were generally larger following the supine exercise compared to the seated exercise (Figure 5.11). Generally, changes from baseline on placebo were increases. The 95% confidence intervals showed statistically significant differences between treatments on day 2 at the 120 W exercise level (-180 to -6 ml) and day 4 at the 50 W exercise level (-93 to -1 ml).

5.5.6.2.9 Cardiac output

Table 5.21 contains the mean change from baseline in cardiac output following seated exercise on days 2, 4 and 6. Table 5.22 contains the point estimates and 95% CI's for the differences between treatments. The mean changes from baseline following tucaresol increased across days 2 to 6 with the maximum increase in cardiac output for all exercise levels occurring on day 6 (Figure 5.12). There was no clear pattern in the mean changes following placebo. The 95% confidence

intervals showed no statistically significant differences between treatments on any day.

Table 5.23 contains the mean change from baseline in cardiac output following supine exercise on days 2, 4 and 6. Table 5.24 contains the point estimates and 95% CI's for the differences between treatments. The mean changes for both treatments were variable although at all days for 0 watts and 50 watts there were decreases in cardiac output following tucaresol. The mean changes from baseline following supine exercise revealed a decrease in cardiac output at most exercise levels and days whereas most mean changes following seated exercise revealed increases in cardiac output (Figure 5.13). The 95% confidence intervals showed no statistically significant differences between treatments.

5.5.6.3 Psychometric tests

There were no trends for differences between treatment groups in any test performance score.

5.5.7 Adverse experiences

All adverse experiences are listed in Table 5.25.

Four volunteers (Subjects 1, 6, 7 and 9) reported no adverse experiences during the study. Three of the subjects received placebo and one (Subject 1) received active drug. Except where specifically mentioned, all adverse experiences reported are for subjects receiving active drug.

Minor gastrointestinal disturbances reported included nausea, vomiting and diarrhoea. In the first cohort of subjects who received each dose in a single fraction, 2 subjects (Subjects 2 and 3) reported diarrhoea. Subject 2 reported diarrhoea after each dose and Subject 3 reported diarrhoea only after the first dose. Although the symptoms caused little discomfort, there was concern that this symptom might unblind the study and hence, it was decided for all further doses to divide each dose into two fractions of approximately equal size and there were no further reports of diarrhoea. Subject 12 reported nausea and vomiting in conjunction with headache (see below) and it appears these symptoms may have been due to migraine.

Headache was reported by Subject 11 (placebo) and Subjects 4, 10 and 12. Prolonged headache was reported on three occasions by Subject 12 and on one occasion associated with tunnel vision, and accompanied by nausea and vomiting. This subject previously had a history of infrequent migraines with aura. These episodes resembled his usual migraine but were more prolonged.

Three subjects (Subjects 2, 5 and 8) on active drug developed a syndrome of adverse experiences characterised by fever, rash and tender cervical lymphadenopathy. Although swelling of the neck nodes was minimal, the tenderness in one subject (Subject 8) was such that he was reluctant to move his head from side to side. These symptoms developed on days 7-10, 2-5 days after dosing had been completed, and so none of the subjects was withdrawn from the study as a result of these adverse experiences. It was initially thought that these

symptoms might represent a viral illness but the three subjects were in separate cohorts, viral serology (see section 5.5.8.3) was negative and no volunteer on placebo was similarly affected. It was concluded that it was likely that these adverse experiences were related to tucaresol. Although symptoms largely subsided within a few days, malaise, a faint rash and some mild tenderness of the neck nodes persisted for up to two weeks. No specific treatment was administered.

Subject 10 reported swelling of the upper lip, tongue and a raised area on the lateral aspect of the trunk and on the foot occurring during the early morning (3 am) of day 16. The symptoms were suggestive of angioedema but there was no abnormality when he was examined at 9 am.

5.5.8 Haematology, Plasma Biochemistry and Viral Serology

5.5.8.1 Haematology

Occasional reticulocyte counts were elevated in each treatment group, but there were no statistically significant differences between treatment groups. There were no trends for changes in any other variable in either treatment group.

5.5.8.2 Plasma Biochemistry

Five volunteers on active drug and two volunteers on placebo had levels of liver enzymes (AST or ALT) outside the normal range during the study period.

Subject 3 (active) had an initial ALT level of 20 U/L and had values above the normal range between days 7 to 14, with a peak level of 82 U/L on day 9. Levels

of 171 and 89 U/L were recorded on days 77 and 84. AST was elevated at 56 U/L on day 9 and 79 U/L on day 77.

Subject 5 (active) had elevated levels of ALT between days 11 and 21 with peak values of 70 U/L on day 11 and 82 U/L on day 18. His baseline level was 17 U/L. AST was elevated at 47 U/L on day 11.

Subject 6 (placebo) had elevated values of ALT between days 3 and 14. His baseline value was at 42 U/L and peak value of 219 U/L was recorded on day 7. AST was elevated between days 5 and 9 with a peak level of 105 U/L on day 7.

Subject 8 had elevated levels of ALT between days 5 and 9 and days 21 and 35. The baseline value was 31 U/L and the peak between days 5 and 9 was 60 U/L on day 7 and the peak value between days 21 and 35 was 75 on day 21. AST levels remained within the normal range.

Subject 9 (active) had elevated levels of ALT between days 7 and 11 with a peak value of 51 U/L on day 7. The baseline value was 42 U/L. AST values remained in the normal range.

Subject 10 (active) had elevated values of ALT between days 7 and 11 and on days 28 and 35. The peak value between days 7 and 11 was 67 U/L on day 9 and subsequently, the peak value was 68 U/L on day 35. Elevated levels of AST were recorded at 48 U/L on day 9 and 59 and 98 U/L on days 28 and 35.

Subject 11 (placebo) had elevated values of ALT on days 5 and 14 with a peak value of 149 U/L on day 9. The baseline value was 22 U/L on day 1. Elevated levels of AST of 58 and 83 U/L were recorded on days 7 and 9 respectively.

Because the elevated levels for subject 6 were regarded as possibly clinically significant, the code for that subject was broken and he was found to be on placebo. He was extensively questioned about possible recent illness, drugs or exposure to chemicals in the workplace but no explanation was found for these elevated liver function tests.

No other abnormalities in clinical biochemistry parameters were considered clinically significant.

5.5.8.3 Viral Serology

There was no evidence of recent infection with Epstein-Barr virus, hepatitis A, influenza A & B, adenovirus, mycoplasma, herpes simplex, psittacosis, measles, mumps, cytomegalovirus, or Q fever.

5.6 **Discussion**

The same trends and relationships observed in the pharmacokinetics in the previous study are reflected in the pharmacokinetics at the higher doses of the present study. Although t_{max} is not well determined in the present study due to the limited once a day sampling, the values do tend to be higher in whole blood compared with plasma, as observed before. The mean dose in the present study of just over 6 g

does not actually result in a higher mean (SD) plasma C_{max} value of 81.4 (4.1) μg/ml) compared with the previous value of 95.8 (26.1) μg/ml from a single dose of 3.6 g, despite higher %MOD and erythrocyte concentrations being achieved in this study. This is due partly to the losses from elimination over the 5 day dosing period but is also due to a higher proportion of tucaresol in the erythrocyte compared to plasma in the present study. The mean erythrocyte C_{max} values for the present and previous studies are 1459 (162) μg/ml and 1035 (67) μg/ml respectively leading to an erythrocyte / plasma ratio of 18.9 compared with 10.8. This continues the trend seen in the previous dose-escalation study for the erythrocyte/plasma ratio to increase with dose.

Plasma, haemolysate and erythrocyte dominant elimination half-lives in the present study are very similar to the values for the highest dose in the previous study with the mean (SD) plasma $t_{½}$ of 257 (37) hours (10.7 d) being much longer than the erythrocyte $t_{½}$ of 157 (29) hours (6.5 d). The effect of this may be seen in the plasma profiles where, due to the longer sampling in the present study, it is apparent that the levels of compound in plasma and erythrocytes gradually approach each other and become equal after around 48 or 55 days after the first dose when the plasma level has fallen to 2-3 μg/ml. In fact, the fall in the erythrocyte / plasma concentration ratio from a mean (SD) of 18.9 (2.4) down to 1 closely parallels the elimination of compound from plasma. The ratio of mean concentration in erythrocytes to mean plasma concentration at each time point is plotted against mean plasma concentration in Figure 5.14. This relationship is approximately linear and presumably reflects the balance between binding to

haemoglobin and plasma proteins both of which involve complex binding patterns with a number of binding sites possessing different affinities for tucaresol, in a similar manner to that demonstrated for valeresol (Merrett *et al*, 1986).

Although the present study involves multiple dosing but not to steady state, it was still appropriate to calculate plasma clearances since both total dose and AUC values should be simply the sum of the individual components assuming linear kinetics. However, the model-independent AUC values for the present study may be less well-determined with the limited daily sampling over the dosing period, and the kinetics in the dose-escalation study did show dose dependencies. Notwithstanding these caveats, the mean (SD) apparent plasma clearance in the present study of 2.77 (0.37) ml/min agrees reasonably well with the mean value for the previous study of 2.27 ml/min at the highest dose.

In the first oral dose-escalation study of tucaresol in healthy volunteers, single doses of tucaresol were used. These doses produced a peak of 19 - 26 %MOD and, at the highest doses (3600 mg), abdominal discomfort was observed. The previous study did not explore the full predicted therapeutic range of %MOD (15-30%) and the present study was designed to allow the predicted therapeutic %MOD range to be assessed.

The use of fractionated loading with tucaresol in this study allowed the target %MOD levels to be accurately titrated. Mean (SD) %MOD achieved was 17.4 (1.4) % on day 2, 27.6 (1.7) % on day 4 and 34 (1.7) % on day 6 compared to the

target of 15%, 25% and 32.5% respectively. The slight overshoot may have been due to the splitting of most doses into two fractions. The previous volunteer study showed that the t_{max} of tucaresol in plasma and erythrocytes was dependent on dose, and the equations used to titrate the doses in this study were based on single doses being administered. These levels of %MOD were initially well tolerated with most frequently reported adverse experience during the residential phase of the study being headache. Diarrhoea, nausea and vomiting occurred at lower doses than in the previous volunteer study and may occur after single doses as low as 2600 mg. The reports of nausea and vomiting on day 1 for subjects on active drug are likely to represent a direct action of tucaresol on the gastrointestinal system. These symptoms are similar to those previously reported with large doses of tucaresol in the first volunteer study. There were no further reports of gastrointestinal symptoms on subsequent days when the volunteers received a lower dose. In both studies, drug was administered fasting and the effect of food on gastrointestinal tolerability of tucaresol or absorption is not known at present.

Three volunteers developed a syndrome of adverse experiences characterised by fever, rash and tender cervical lymphadenopathy. It appears likely that these symptoms are related to tucaresol and the pattern of symptoms suggests an immunologically mediated reaction or drug allergy. Similar adverse experiences were not reported in the previous volunteer study, despite peak plasma concentrations in the current study being slightly lower than in the previous study (see below). It is possible that multiple dosing or the attainment of higher erythrocyte drug concentrations may have been factors in the occurrence of these

adverse experiences in the current study. However there must be other factors involved since only 3 out of 8 subjects developed the adverse experiences despite the attainment of similar achieved concentrations among all subjects, due to the titrated dosing design. It is also possible that these adverse experiences did not occur in the previous study because of the small number of subjects (4) at each dose, or because volunteers had gradually increasing single doses over a period of months.

Compared to the baseline exercise on day 0, heart rate following exercise progressively increased after tucaresol administration but was unchanged after placebo. These changes were more evident at the highest work rates and following supine exercise rather than seated. The greater drug-related increase in heart rate after exercise compared to at rest is consistent with a dose-related increase in exercise heart rates in volunteers receiving a 2 hour infusion of valeresol (Nicholls *et al*, 1989a). Increased demand for oxygen supply under exercise is more likely to require a greater increase in heart rate to maintain oxygen delivery with modified haemoglobin than at rest because the reduced oxygen delivery per unit volume of blood at higher %MOD levels may become limiting. The increases in heart rate were not accompanied by similar changes in cardiac output. Although the BoMed device has been well validated to detect changes in stroke volume and cardiac output at rest (Northridge *et al*, 1990; Pincomb *et al*, 1993; Castor *et al*, 1994; Ng *et al*, 1991; Thomas, 1991), and reasonable results obtained under exercise (Miles *et al*, 1993), its accuracy under exercise has been questioned (Smith *et al*, 1988). This is probably due to muscle activity and respiration affecting the thoracic impedance

signal with which the device calculates stroke volume. The modest changes in heart rate suggest that the increase in oxygen affinity is acutely well tolerated by the cardiovascular system.

The study produced no evidence that psychometric performance was impaired under resting conditions at up to 38 %MOD. It is unlikely that an increase in oxygen affinity would significantly affect cerebral function because of cerebral auto-regulation of blood flow based on demand. However, the power of the study to detect changes in psychometric performance due to tucaresol was not high.

5.7 Conclusions

- Titrated loading doses as used in this study achieve target %MOD levels with a slight bias towards overshoot but with good precision.

- Pharmacokinetics of tucaresol after a fractionated loading dose are complex but similar to those reported after single doses.

- Minor gastrointestinal symptoms occur with doses over 2000 mg in the fasting state.

- Fever, rash and tender cervical lymphadenopathy in three out of eight volunteers suggests drug allergy.

- Haemoglobin modification up to 38% produces slight increases in heart rate under moderate exercise but is generally well tolerated by the cardiovascular system.

- Haemoglobin modification up to 35% produces no gross changes in psychometric performance.

TABLES

Table 5.1 Volunteer demographics and treatment allocation

Subject No.	Age (y)	Sex	Weight (kg)	Height (m)	Treatment
1	20	M	61.1	1.86	Placebo
2	20	M	73.0	1.78	Tucaresol
3	31	M	83.1	1.84	Tucaresol
4	21	M	73.2	1.83	Tucaresol
5	31	M	89.5	1.84	Tucaresol
6	33	M	82.2	1.82	Placebo
7	29	M	75.7	1.78	Placebo
8	22	M	76.8	1.87	Tucaresol
9	28	M	98.2	1.81	Tucaresol
10	34	M	78.2	1.82	Tucaresol
11	40	M	78.1	1.83	Placebo
12	25	M	81.8	1.86	Tucaresol
Mean	27.8	-	79.2	1.83	-
SD	6.4	-	9.1	1.83	-
Minimum	20	-	61.1	1.78	-
Maximum	40	-	98.2	1.87	-

Table 5.2 Individual doses of tucaresol (mg)

Subject No.	Tucaresol dose			Total
	Day 1	Day 3	Day 5	
2	2600	1600	600/800	5600
3	2800	2000	800/1000	6600
4	2600	1000/1200	600/800	6200
5	3000	1200/1200	400/400	6200
8	1200/1400	400/800	600/800	5200
9	1600/1800	800/1200	800/800	7000
10	1200/1600	800/800	600/800	5800
12	1200/1600	800/800	800/800	6000
Mean	2825	1825	1425	6075
SD	271	392	292	565

The two fractions of a split dose are separated by "/".

Table 5.3 Summary of pharmacokinetic and %MOD data

Subject No	Dose (mg)	Plasma						Erythrocyte				%MOD		
		C_{max} $\mu g\ ml^{-1}$	t_{max} h	$AUC_{0-\infty}$ $h.\mu g\ ml^{-1}$	$t_{1/2}$ h	CL/F $ml\ min^{-1}$	V_z/F l	C_{max} $\mu g\ ml^{-1}$	t_{max} h	$AUC_{0-\infty}$ $h.\mu g\ ml^{-1}$	$t_{1/2}$ h	Max. %MOD	t_{max} h	$t_{1/2}$ h
2	5556	85.7	120	42844	307	2.16	57.4	1540	120	593178	214	34	120	199
3	6555	83.1	120	37849	247	2.89	61.8	1670	120	501109	149	34	120	187
4	6147	83.2	96	29982	199	3.42	58.8	1195	120	353930	136	33	120	168
5	6158	77.3	96	34037	294	3.02	76.8	1458	96	484546	151	34	120	216
8	5158	76.4	96	34679	271	2.48	58.1	1371	144	476729	127	32	120	209
9	6953	81.9	120	41043	284	2.82	69.3	1638	96	582369	178	38	120	193
10	5760	76.9	120	35988	212	2.67	48.9	1308	120	410120	165	33	120	162
12	5964	86.9	120	36835	241	2.70	56.3	1490	120	486425	133	35	144	187
Mean	6031	81.4	111	36657	257	2.77	60.9	1459	117	486051	157	34.1	122.9	190
SD	563	4.1	12.5	4049	39	0.37	8.6	162	15	79544	29	1.8	8.5	19

Table 5.4 Summary statistics for %MOD at each assessment time						
Day	Minimum	Maximum	Mean	SD	Median	N
1	0	10	4.5	4.84	4.0	8
2	15	20	17.8	1.51	17.5	8
3	14	24	20.5	3.85	22.0	8
4	26	33	28.0	2.61	27.5	6
5	26	33	28.5	2.93	27.0	8
6	28	38	33.1	2.80	33.5	8
7	27	34	31.8	2.55	33.0	8
8	32	35	33.5	2.12	33.5	2
9	21	31	25.9	3.23	26.5	8
11	16	28	21.8	4.03	22.0	8
14	15	23	18.0	2.78	17.0	8
17	8	18	13.4	2.97	13.5	8
21	7	12	9.3	1.67	9.5	8
28	0	6	2.0	2.78	0	8

Table 5.5 Mean change in systolic blood pressure from baseline - seated exercise (mmHg)

Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	-4.6	-3.0	-7.3	-4.2	-4.3	-6.0
50	2.1	-5.8	-7.1	2.3	-3.5	-3.8
85	-0.6	-0.4	-8.1	-3.0	-5.3	-4.5
120	-2.6	-7.5	-16.1	3.5	-2.3	-2.5

Table 5.6 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in systolic blood pressure - seated exercise (mmHg)

Exercise level W	Day 2	Day 4	Day 6
0	-0.4 (-26.5, 25.8)	1.3 (-17.5, 20.0)	-1.3 (-16.7, 14.2)
50	-0.1 (-15.9, 15.6)	-2.3 (-18.2, 13.7)	-3.4 (-20.6, 13.8)
85	2.4 (-15.6, 20.4)	4.9 (-13.2, 22.9)	-3.6 (-21.0, 13.7)
120	-6.1 (-21.1, 8.9)	-5.3 (-24.0, 13.5)	-13.6 (-33.6, 6.4)

Table 5.7 Mean change in systolic blood pressure from baseline - supine exercise (mmHg)

Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	3.4	-0.9	-9.1	4.0	1.0	3.8
50	5.1	0.0	-0.9	1.0	2.3	5.3
85	8.6	1.1	-1.1	-8.0	-2.0	2.5
120	5.4	7.6	2.4	-8.0	-9.5	-9.8

Table 5.8 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in systolic blood pressure - supine exercise (mmHg)

Exercise level W	Day 2	Day 4	Day 6
0	-0.6 (-13.5, 12.2)	-1.9 (-21.1, 17.4)	-12.9 (-31.7, 5.9)
50	4.1 (-14.1, 22.3)	-2.3 (-22.6, 18.1)	-6.1 (-30.1, 17.9)
85	16.6 (2.0, 31.2)	3.1 (-14.3, 20.6)	-3.6 (-26.0, 18.7)
120	13.4 (0.1, 26.7)	17.1 (4.2, 30.1)	12.2 (-5.2, 29.5)

Table 5.9 Mean change in diastolic blood pressure from baseline - seated exercise (mmHg)

Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	0.6	-2.5	-4.3	-2.5	2.3	0.5
50	-5.3	-2.8	-5.6	3.3	-2.8	0.3
85	4.1	-2.0	-2.1	2.0	2.8	-0.5
120	-3.0	-6.6	-10.5	5.5	-2.0	-1.5

Table 5.10 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in diastolic blood pressure - seated exercise (mmHg)

Exercise level W	Day 2	Day 4	Day 6
0	3.1 (-7.1, 13.3)	-4.8 (-10.9, 1.4)	-4.8 (-13.5, 4.0)
50	-8.5 (-16.7, -0.3)	0.0 (-9.5, 9.5)	-5.9 (-12.7, 1.0)
85	2.1 (-6.5, 10.8)	-4.8 (-12.8, 3.3)	-1.6 (-12.3, 9.1)
120	-8.5 (-18.6, 1.6)	-4.6 (-12.6, 3.4)	-9.0 (-16.4, -1.6)

Table 5.11 Mean change in diastolic blood pressure from baseline - supine exercise (mmHg)

Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	4.3	1.1	-0.3	1.5	-2.0	-1.3
50	6.3	-2.4	-1.1	1.3	-1.8	-2.5
85	1.9	-1.3	-3.9	-3.8	-4.8	-5.3
120	0.3	-1.5	-1.9	-4.0	-3.0	-4.8

Table 5.12 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in diastolic blood pressure - supine exercise (mmHg)

Exercise level W	Day 2	Day 4	Day 6
0	2.8 (-4.7, 10.2)	3.1 (-3.5, 9.7)	1.0 (-7.8, 9.8)
50	5.0 (-2.7, 12.7)	-0.6 (-7.5, 6.2)	1.4 (-4.6, 7.3)
85	5.6 (-3.8, 15.0)	3.5 (-8.8, 15.8)	1.4 (-9.0, 11.8)
120	4.3 (-5.7, 14.20	1.5 (-10.4, 13.4)	2.9 (-7.5, 13.3)

Table 5.13 Mean change in heart rate from baseline - seated exercise (bpm)						
Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	6.6	11.5	16.0	-1.4	13.0	10.8
50	11.1	16.2	23.6	2.5	5.7	6.6
85	9.4	19.9	27.2	4.2	7.0	5.0
120	8.8	21.7	28.0	1.9	2.0	4.8

Table 5.14 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in heart rate - seated exercise (bpm)			
Exercise level W	Day 2	Day 4	Day 6
0	8.1 (-3.0, 19.1)	-1.5 (-15.7, 12.7)	5.2 (-9.4, 19.7)
50	8.6 (-7.4, 24.5)	10.5 (-6.9, 27.9)	17.0 (-1.4, 35.4)
85	5.1 (-15.1, 25.4)	12.8 (-3.2, 28.9)	22.1 (3.4, 40.8)
120	6.9 (-15.1, 28,8)	19.1 (2.7, 35.6)	23.3 (8.4, 38.1)

Table 5.15 Mean change in heart rate from baseline - supine exercise (bpm)						
Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	2.9	7.0	6.6	-0.2	2.5	4.2
50	2.3	10.6	10.1	-4.3	-0.9	-6.7
85	5.8	10.3	14.6	-0.5	4.8	-1.6
120	12.3	18.4	19.9	-3.4	-0.7	-4.7

Table 5.16 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in heart rate - supine exercise (bpm)			
Exercise level W	Day 2	Day 4	Day 6
0	3.1 (-7.9, 14.10)	4.5 (-8.4, 17.5)	2.4 (-14.4, 19.2)
50	6.6 (-5.5, 18.8)	11.5 (3.4, 19.7)	16.8 (0.4, 33.3)
85	6.3 (-6.7, 19.2)	5.4 (-5.2, 15.9)	16.1 (8.3, 23.9)
120	15.7 (9.2, 22.2)	19.1 (10.8, 27.4)	24.5 (12.6, 36.5)

Table 5.17 Mean change in stroke volume from baseline - seated exercise (ml)

Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	-17.4	-22.2	-17.7	6.0	-36.7	-25.4
50	-18.5	-10.6	-17.6	27.7	16.3	-1.2
85	-12.8	-1.6	-7.8	20.2	-15.7	18.6
120	-14.0	-11.9	-1.1	22.4	16.3	37.9

Table 5.18 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in stroke volume - seated exercise (ml)

Exercise level W	Day 2	Day 4	Day 6
0	-23.4 (-47.7, 0.9)	14.6 (-22.7, 51.8)	7.7 (-34.3, 49.7)
50	-46.2 (-102.0, 9.6)	-26.9 (-100.3, 46.5)	-16.3 (-77.7, 45.0)
85	-33.0 (-85.5, 19.5)	14.1 (-61.9, 90.1)	-26.5 (-98.7, 45.8)
120	-36.4 (-102.6, 29.9)	-28.2 (-108.5, 52.1)	-39.0 (-130.6, 52.6)

Table 5.19 Mean change in stroke volume from baseline - supine exercise (ml)						
Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	-23.4	-40.0	-17.6	30.6	2.7	1.6
50	-35.9	-28.3	-36.4	28.7	18.6	-2.2
85	-27.4	-14.9	-46.3	12.9	-2.9	-12.7
120	-45.2	-40.2	-48.9	47.6	35.1	13.1

Table 5.20 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in stroke volume - supine exercise (ml)			
Exercise level W	Day 2	Day 4	Day 6
0	-53.9 (-131.7, 23.9)	-42.7 (-101.3, 15.9)	-19.3 (-80.0, 41.4)
50	-64.6 (-149.0, 19.8)	-46.9 (-93.2, -0.7)	-34.2 (-100.5, 32.1)
85	-40.2 (-136.2, 55.7)	-12.1 (-90.2, 66.1)	-33.5 (-161.3, 94.2)
120	-92.8 (-179.2, -6.3)	-75.3 (-160.6, 10.0)	-62.0 (-138.1, 14.1)

Table 5.21 Mean change in cardiac output from baseline - seated exercise (l/min)

Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	-0.6	-0.3	0.5	0.3	-1.1	-0.4
50	0.2	1.9	2.5	2.8	0.7	1.3
85	0.6	3.6	4.8	3.1	-0.6	3.3
120	-0.0	2.7	6.1	3.2	3.1	8.8

Table 5.22 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in cardiac output - seated exercise (l/min)

Exercise level W	Day 2	Day 4	Day 6
0	-0.9 (-2.5, 0.7)	0.8 (-2.4, 4.0)	0.9 (-2.2, 4.0)
50	-2.7 (-7.0, 1.7)	1.2 (-5.6, 7.9)	1.2 (-4.2, 6.5)
85	-2.5 (-7.3, 2.2)	4.2 (-2.8, 11.1)	1.1 (-6.6, 8.9)
120	-3.2 (-8.4, 2.1)	-0.3 (-9.6, 8.9)	-2.7 (-16.9, 11.5)

Table 5.23 Mean change in cardiac output from baseline - supine exercise (l/min)

Exercise level W	Tucaresol (N=8)			Placebo (N=4)		
	Day 2	Day 4	Day 6	Day 2	Day 4	Day 6
0	-1.2	-1.7	-0.6	1.4	0.4	0.8
50	-2.3	-0.3	-0.5	0.9	1.6	-1.5
85	-1.3	1.1	-1.3	0.6	0.7	-1.8
120	-1.6	0.6	0.1	4.9	3.9	0.7

Table 5.24 Point estimates and (95%CI's) for the difference between treatments (Tucaresol-Placebo) in change from baseline in cardiac output - supine exercise (l/min)

Exercise level W	Day 2	Day 4	Day 6
0	-2.7 (-6.2, 0.9)	-2.1 (-5.8, 1.6)	-1.3 (-5.8, 3.1)
50	-3.2 (-10.0, 3.6)	-1.8 (-7.0, 3.3)	1.0 (-3.5, 5.4)
85	-1.9 (-8.2, 4.4)	0.4 (-5.1, 5.9)	0.5 (-10.7, 11.7)
120	-6.5 (-13.8, 0.8)	-3.3 (-10.7, 4.0)	-0.6 (-9.6, 8.4)

Table 5.25 Adverse experiences

Subject No	Study Day	Adverse Experience	Treatment
Placebo subjects			
1	-	None	-
6	7	Elevated transaminases	None
7	-	None	-
11	3	Headache	None
	6	Tender node R axilla	None
	7	Headache	None
Tucaresol subjects			
2	1	Diarrhoea	None
	7	Headache, fever, rash, tender cervical lymphadenopathy	Paracetamol
3	1	Diarrhoea	None
	11	Stuffy nose	None
	31	Flu-like symptoms	None
	65	Fever, malaise, diarrhoea	None
4	5,6	Headache	None
5	1	Nausea, vomiting, diarrhoea	None
	8	Fever, sore neck, malaise	Paracetamol
	9	Morbilliform rash, tender cervical lymphadenopathy	Paracetamol

8	8,9	Headache	None
	10	Headache, fever, tender cervical lymphadenopathy	Paracetamol
9	-	None	-
10	3,5	Headache	None
	16	Angioedema of lip, tongue, trunk and foot	None
12	1	Migraine	None
	5	Migraine	Paracetamol
	7	Migraine	Paracetamol

FIGURES

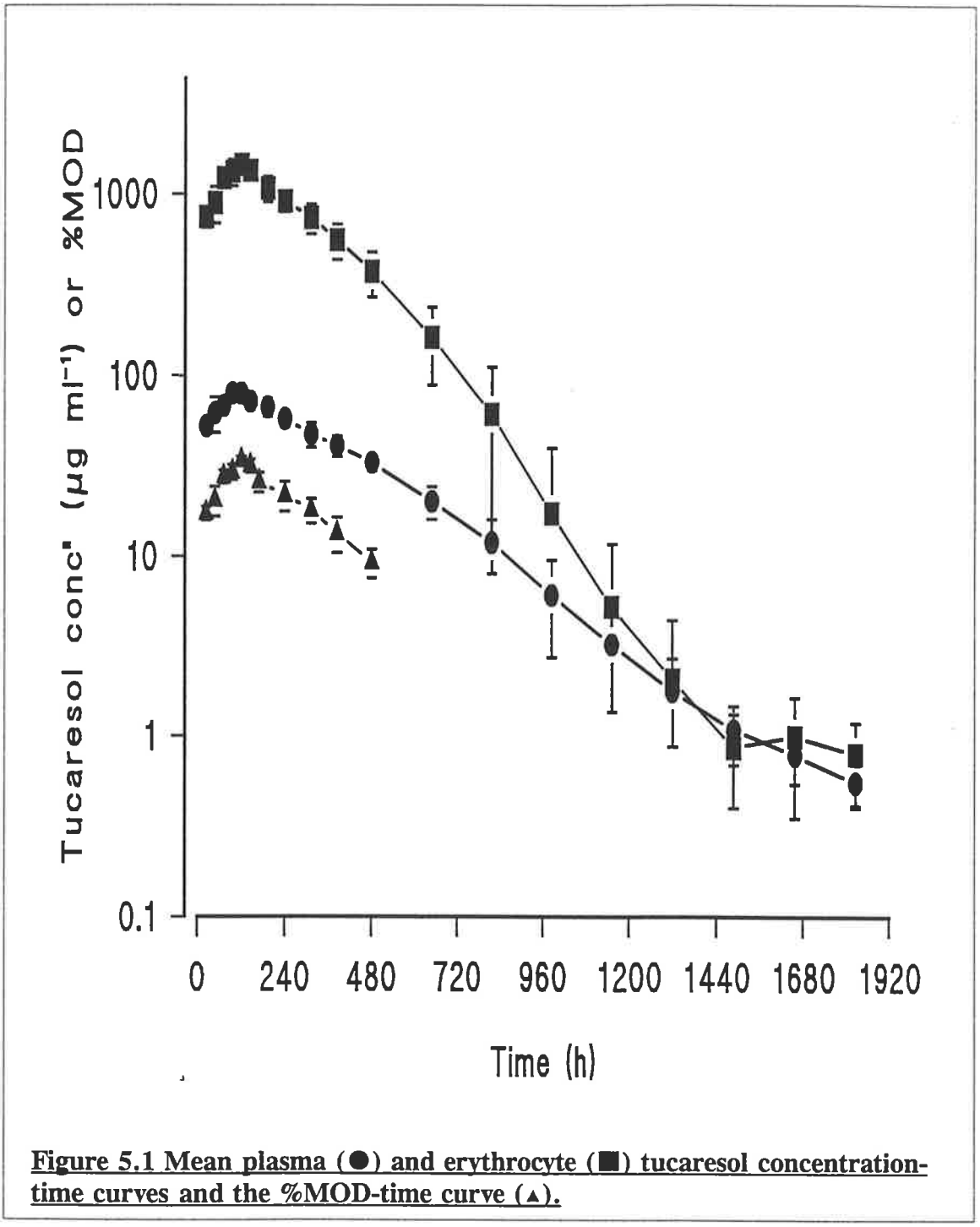


Figure 5.1 Mean plasma (●) and erythrocyte (■) tucaresol concentration-time curves and the %MOD-time curve (▲).

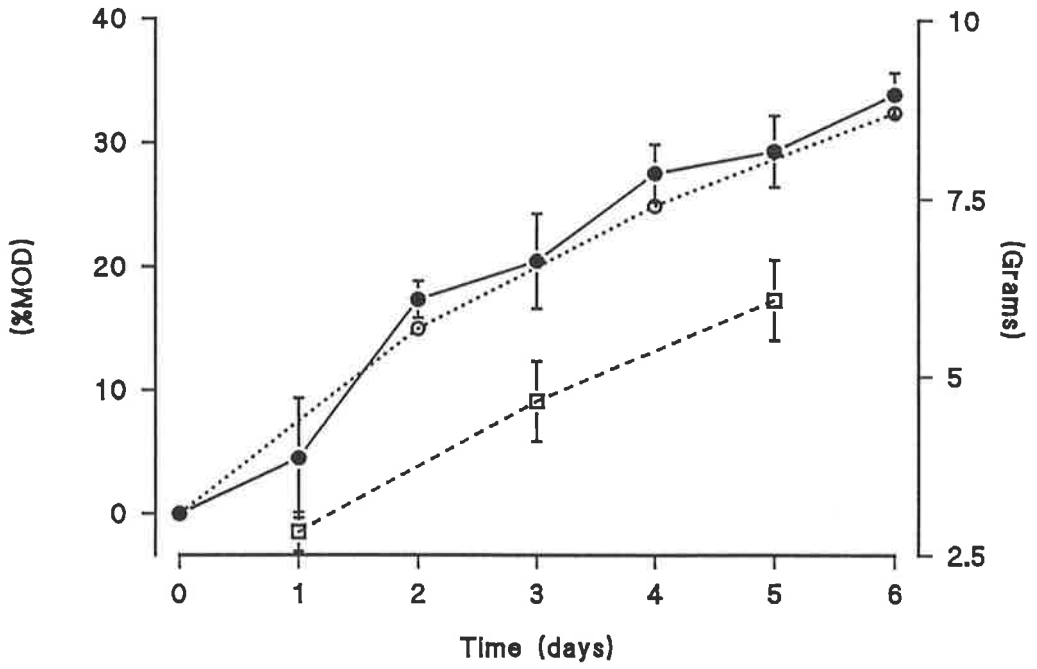


Figure 5.2 A comparison of target (○) and achieved (●) %MOD related to dose (□).

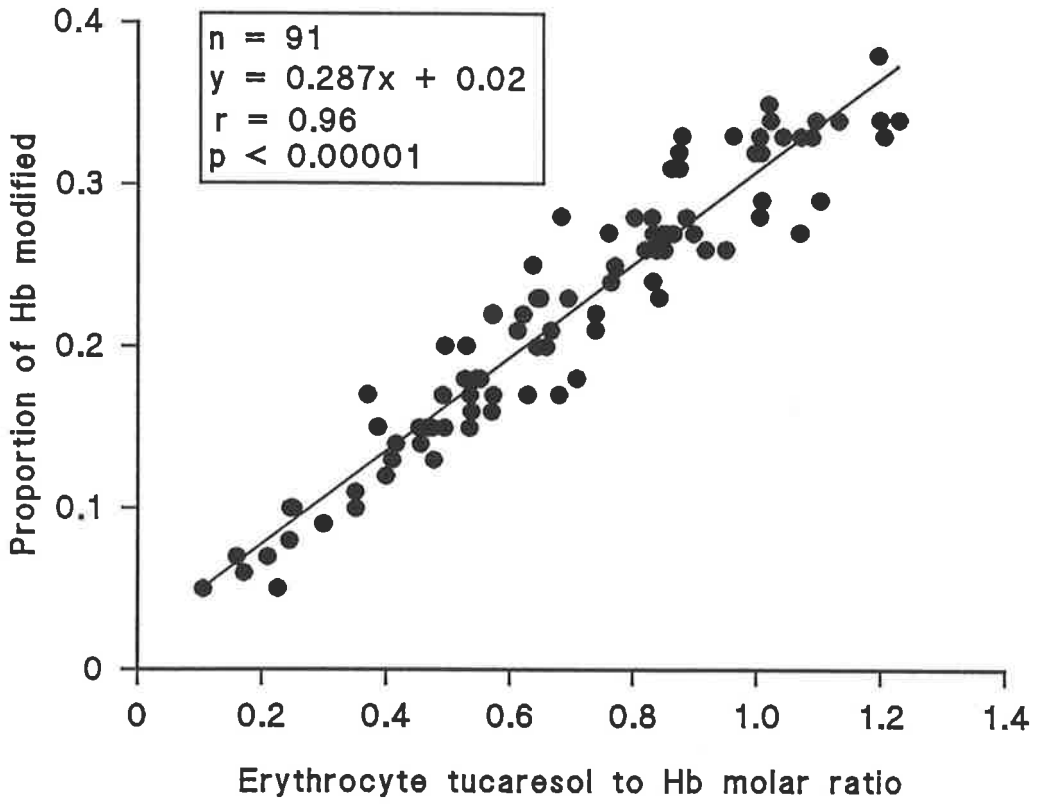


Figure 4.4 Proportion of modified Hb (%MOD / 100) as a function of erythrocyte tucaresol to Hb molar ratio.

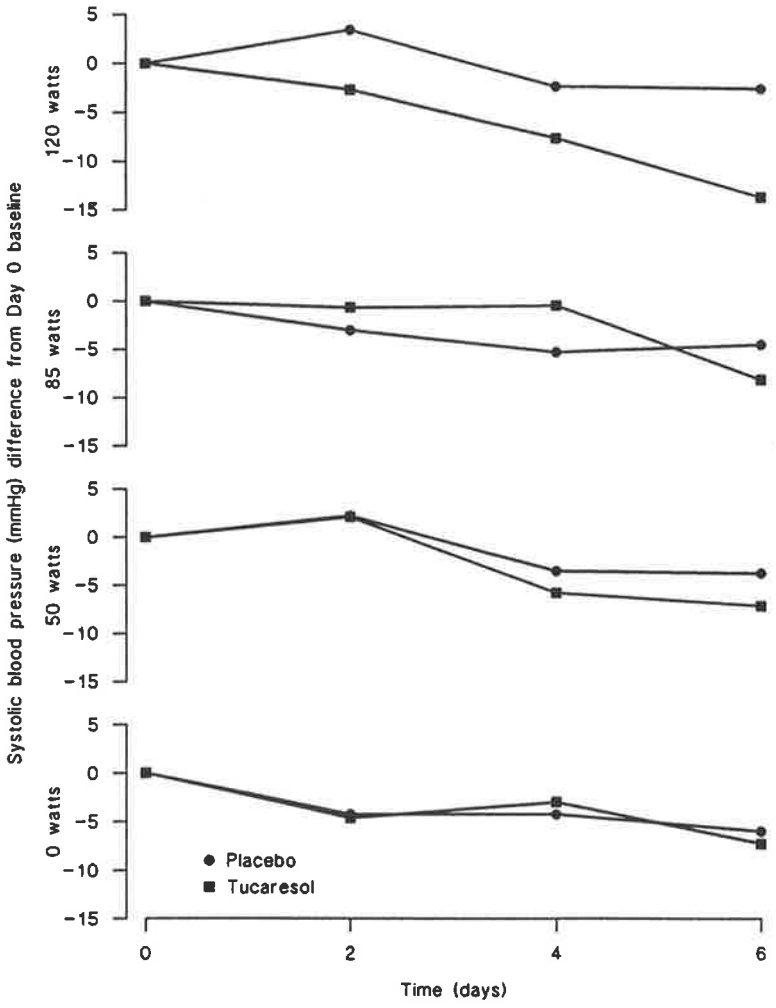


Figure 5.4 Mean changes from baseline in systolic blood pressure - seated exercise.

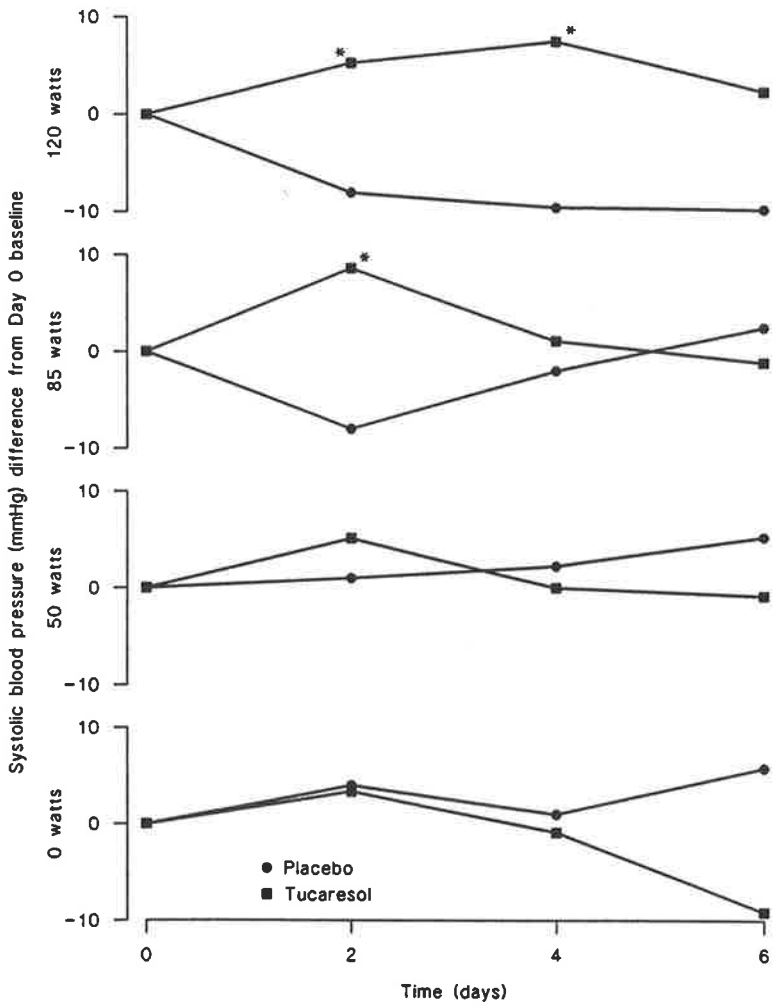


Figure 5.5 Mean changes from baseline in systolic blood pressure - supine exercise. * = $p < 0.05$

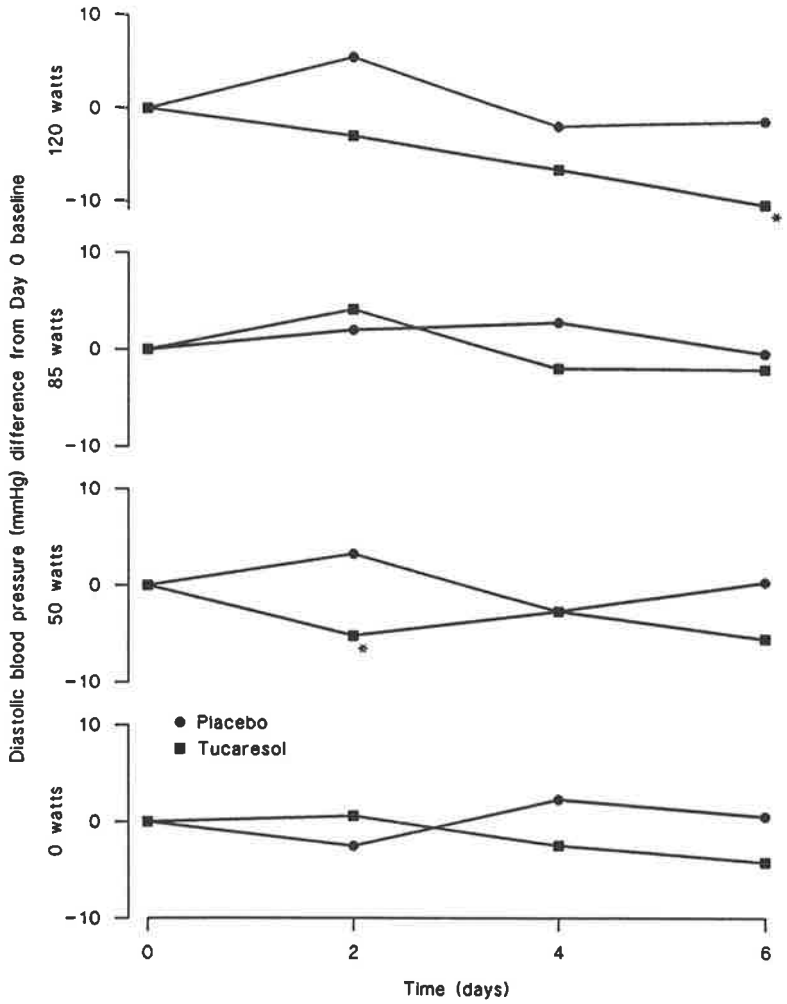


Figure 5.6 Mean changes from baseline in diastolic blood pressure - seated exercise. * = $p < 0.05$

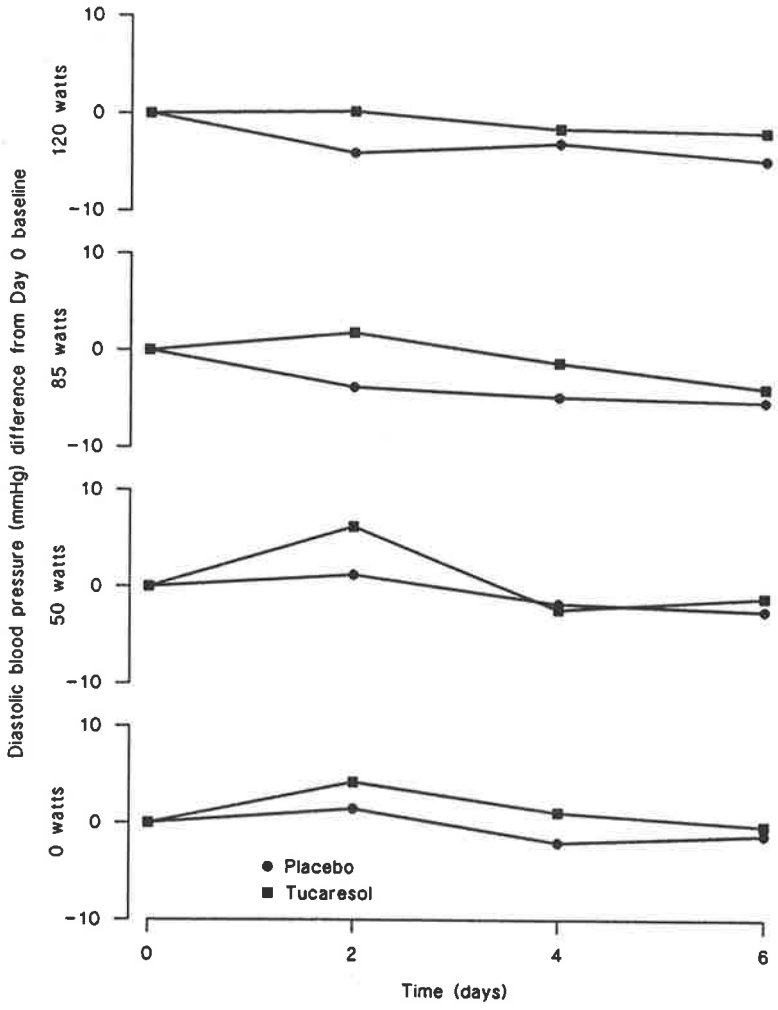


Figure 5.7 Mean changes from baseline in diastolic blood pressure - supine exercise.

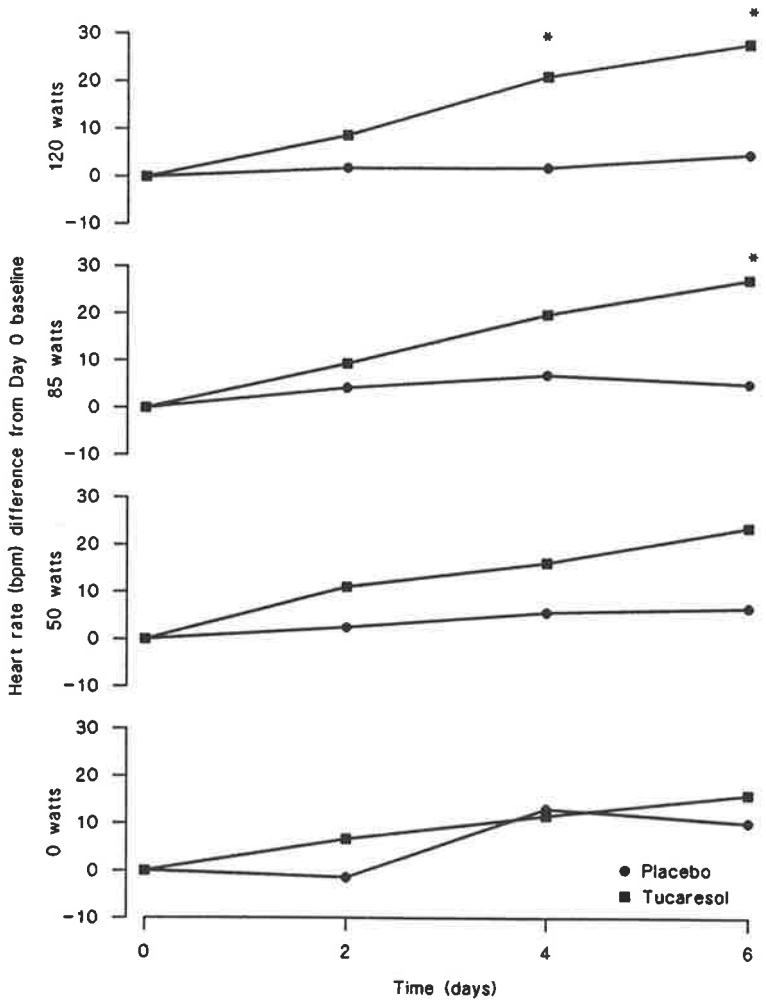


Figure 5.8 Mean changes from baseline in heart rate - seated exercise. * = $p < 0.05$.

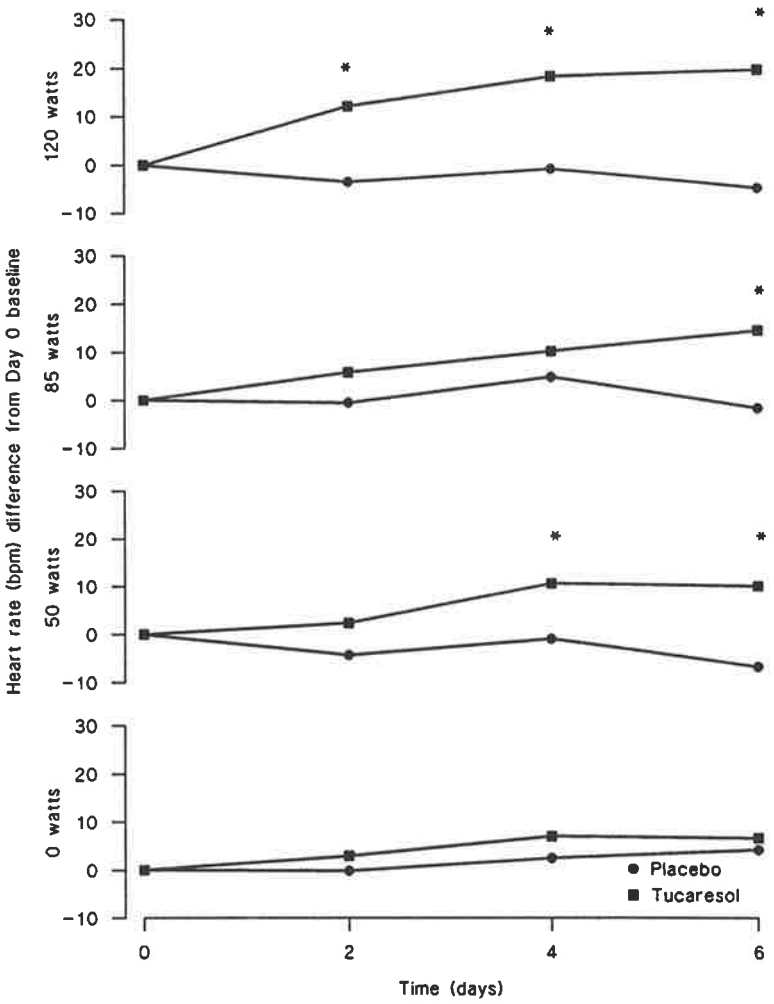


Figure 5.9 Mean changes from baseline in heart rate - supine exercise. * = $p < 0.05$

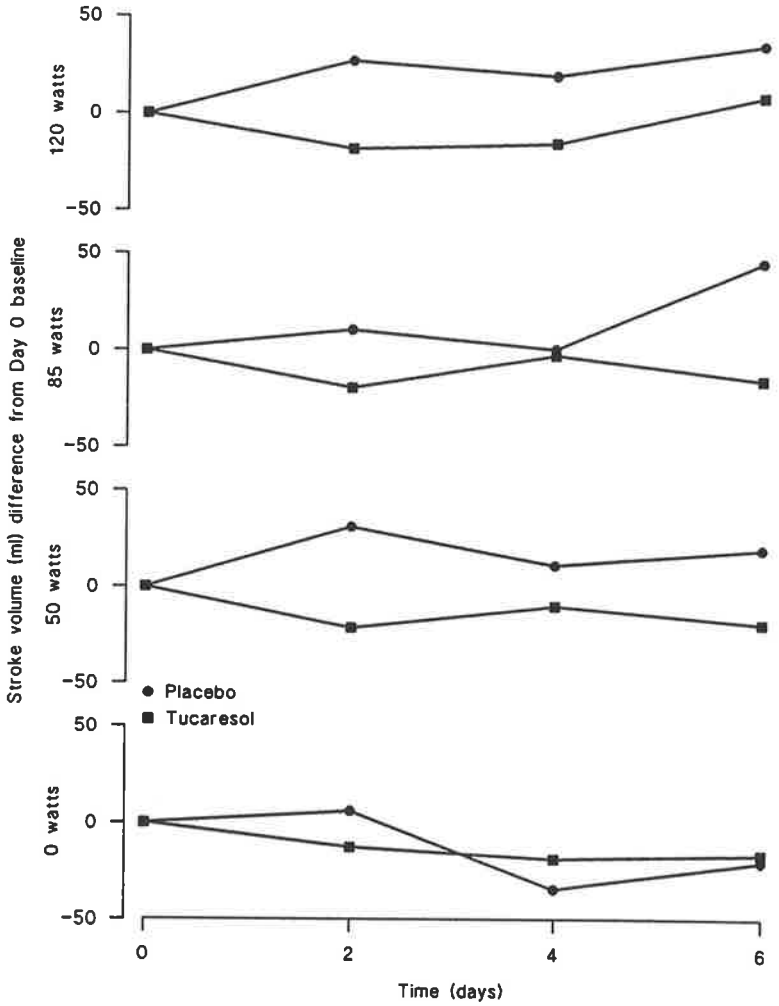


Figure 5.10 Mean changes from baseline in stroke volume - seated exercise.

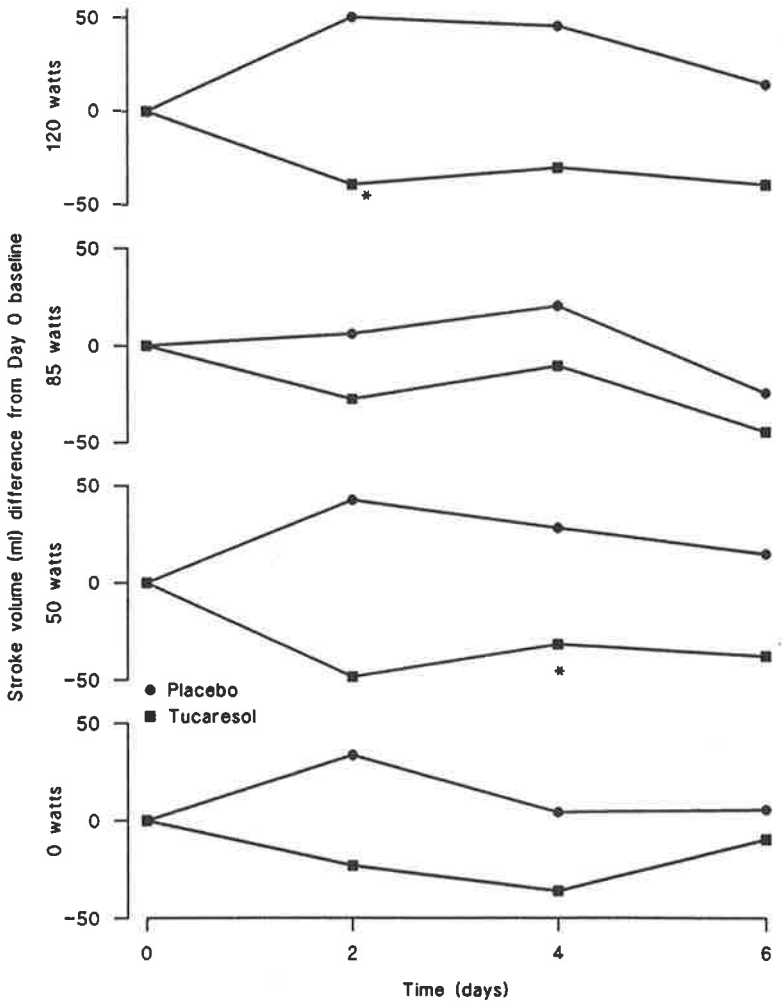


Figure 5.11 Mean changes from baseline in stroke volume - supine exercise. * = $p < 0.05$

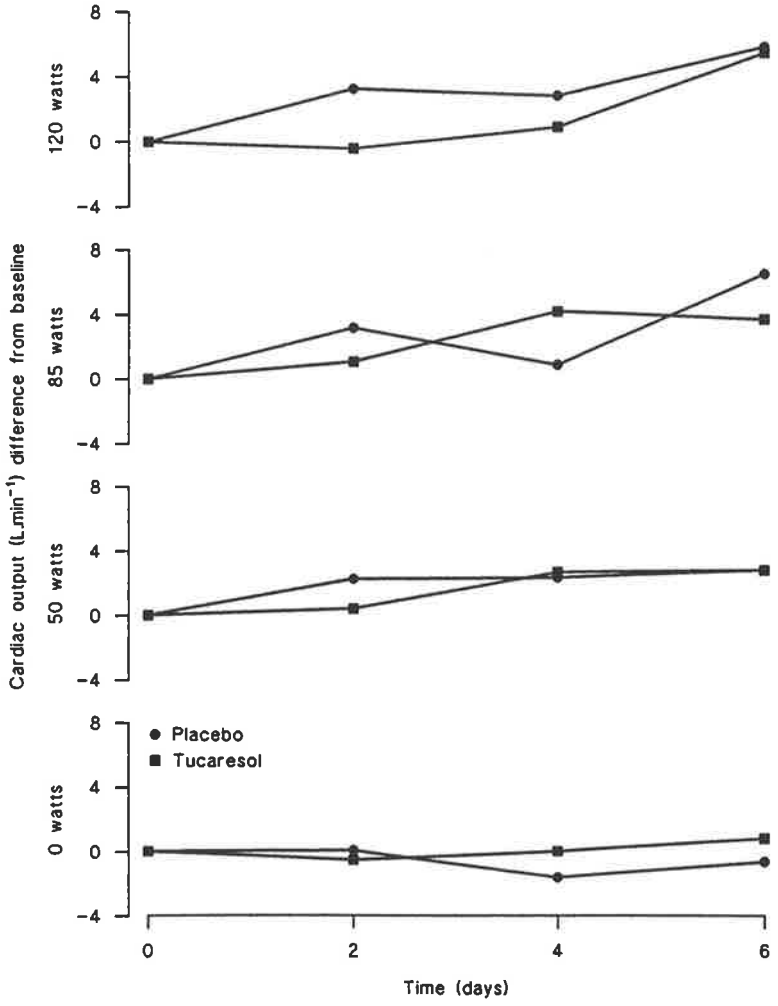


Figure 5.12 Mean changes from baseline in cardiac output - seated exercise.

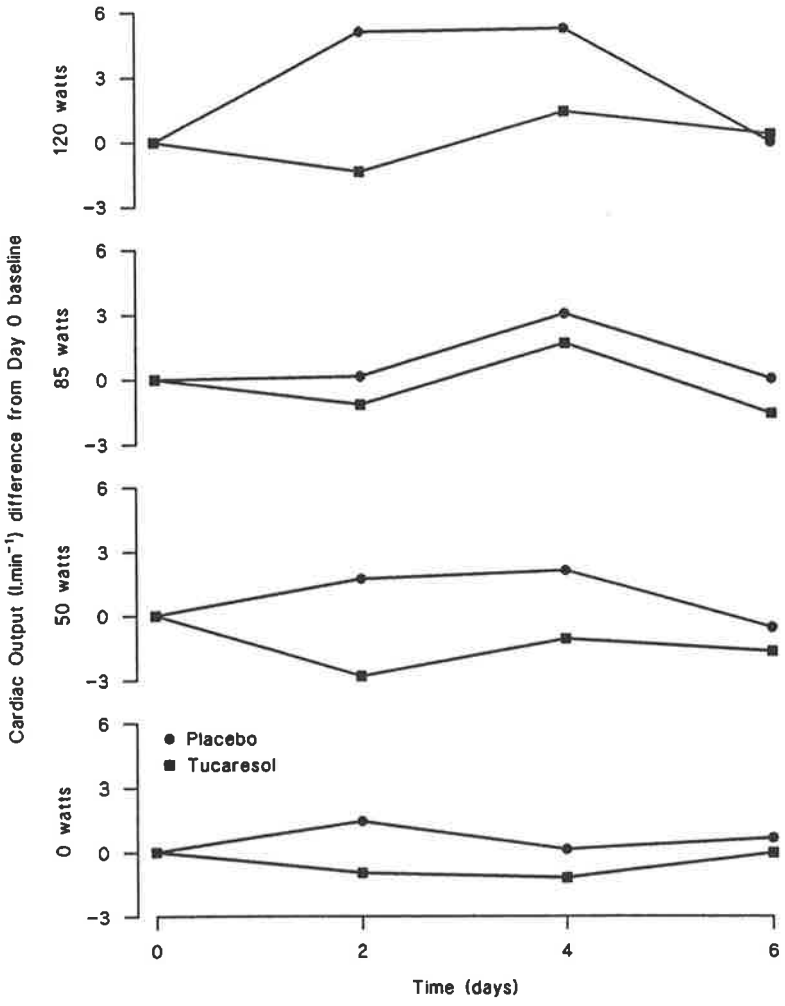


Figure 5.13 Mean changes from baseline in cardiac output - supine exercise.

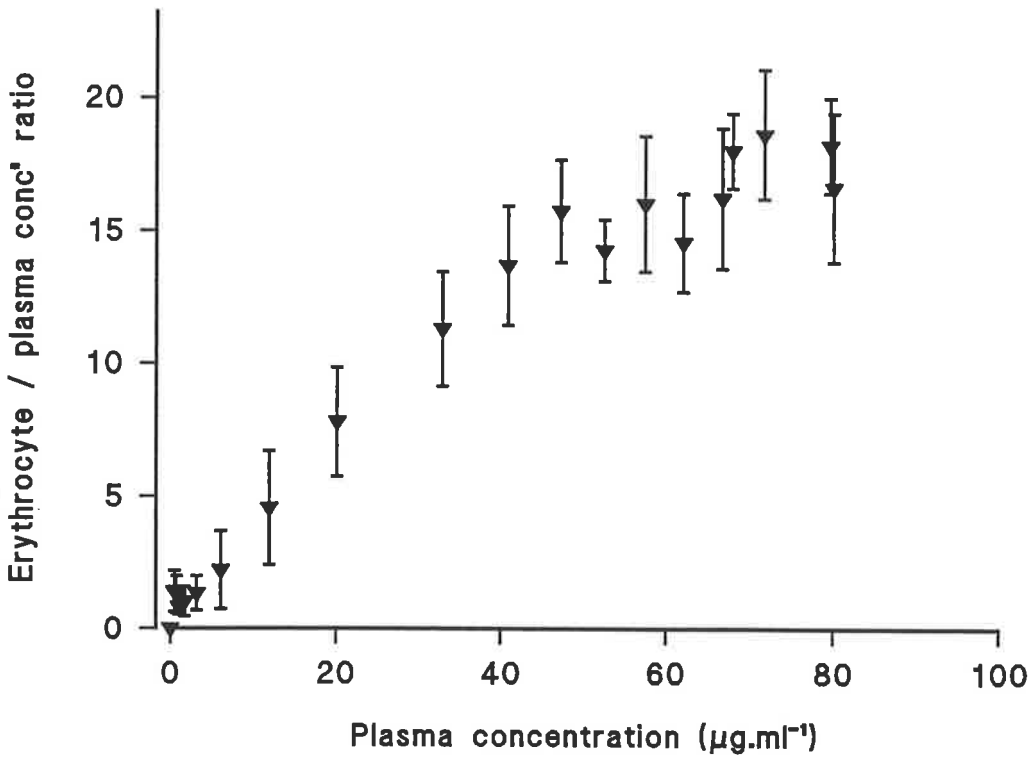


Figure 5.14 Erythrocyte / plasma ratio of tucaresol as a function of plasma concentration.

CHAPTER 6

THE PHARMACOKINETICS, TOLERABILITY AND EFFECTS ON HAEMOLYSIS OF MULTIPLE DOSES OF TUCARESOL IN PATIENTS WITH SICKLE CELL DISEASE

6.1 **Introduction**

The multiple dose study in healthy volunteers described in the last chapter demonstrated that haemoglobin modification to a greater extent than that thought to be needed for therapy in sickle cell disease was well tolerated haemodynamically and by the brain. However, the development of adverse experiences suggestive of drug allergy occurring in three of eight volunteers raised significant concern as to whether clinical development of tucaresol in patients should proceed any further, although it was clearly inappropriate to perform further multiple dose studies in healthy volunteers. Points in favour of continuing development included:

- there was no alternative prophylactic therapy for the painful, debilitating and serious consequences of sickle cell disease;
- the adverse experiences only occurred when >30% MOD had been achieved, and lower %MOD with a consequently reduced body burden of drug might be clinically effective and might reduce the risk of drug allergy;
- although the adverse experiences were uncomfortable for the volunteers, there was no evidence of serious toxicity and there were no sequelae; the intensity of the adverse experiences was similar to that of other acceptable therapies for serious diseases (e.g. interferon);
- it was possible that the adverse experiences could be managed by a temporary reduction or cessation of dosing, which would not be a major problem for a drug intended for long-term therapy.

The major point against proceeding was that clinical consequences of drug allergy can progress to fatal hepatic necrosis (eg phenytoin) or toxic epidermal necrolysis (eg carbamazepine). Although there was no evidence of such serious toxicity in the volunteer study, this might be because dosing with tucaresol was discontinued before the onset of the adverse experiences and it was possible that the outcome of an episode of drug allergy might be more serious if drug administration were continued after the onset of symptoms. In addition to extensive discussions within the company on whether to proceed, we invited the opinion of an external consultant, Professor Bellingham (Professor of Haematology, Kings College Hospital, London SE5) with extensive clinical and research experience in sickle cell disease. Professor Bellingham's opinion comprised not only his personal advice but was also based on discussions with patients as to whether they were prepared to risk the development of adverse experiences such as those we had seen in volunteers, as part of the development of a therapy for the disease. As a result of these discussions, we decided to proceed to further cautious development of tucaresol in patients, and this chapter describes the first study of the effects of systemic administration of tucaresol in patients with sickle cell disease.

6.2 Objectives

The specific objectives of the study were, in stable patients with sickle cell disease, receiving tucaresol for 10 days, at doses producing 20-25 %MOD :

- to examine the effect on markers of haemolysis;
- to examine the tolerability;

- to examine the pharmacokinetics;
- to examine the effects on resting and exercise heart rate and blood pressure.

6.3 Study Design

6.3.1 General

The study was of double-blind placebo-controlled design. It was intended to enrol 18-24 stable patients with sickle cell disease of both sexes and twice as many patients were to receive active drug as placebo. The study consisted of four phases : screening, baseline, treatment and follow-up.

The screening phase consisted of a single outpatient visit during which patient suitability was assessed. During the baseline phase a haematological evaluation was carried out, including an assessment of erythrocyte survival. The treatment phase consisted of 11 days during which the patient resided at the Investigation Unit and during which tablets were administered daily apart from the day of admission. It was intended to give a fractionated loading dose over 3-5 days to reach 20-25 %MOD and then a maintenance dose to maintain this level of %MOD for a total period of 10 days. During the treatment phase the cardiovascular response to moderate graded exercise and a further haematological evaluation was performed. The follow-up phase consisted of 4 outpatient visits at weekly intervals with a haematological evaluation on the last visit.

6.3.2 Design considerations

6.3.2.1 Selection of dosing regimen

The dosing schedule was designed so that patients would reach the target range of 20-25 %MOD within 3-5 days dosing and maintain %MOD within this range for a total of 10 daily doses. This range is in the middle of the anticipated therapeutic range of 15-30 %MOD and is lower than the range in the previous volunteer study in which significant adverse experiences occurred. It was hoped that the lower range of target %MOD might avoid similar adverse experiences. Loading was performed over several days to avoid the gastrointestinal effects of large single doses and enable titration of the dose to achieve the required %MOD so that significant under- or over-shoot should have been avoided (see dosing). One caveat in this design was that doses were selected on the basis of data from healthy volunteers and the lower haematocrits of patients may have resulted in different dose requirements. However previous experience with valeresol (see Chapter 3) suggested that there was no major difference in the relationship between dose and %MOD between healthy volunteers and patients. Body weight was taken into account in selecting doses because of the trend for a relationship between peak %MOD and body weight in the first volunteer study, after correcting for dose.

6.3.2.2 Statistical considerations

As this study was exploratory in nature the number of subjects was determined by the likely number of available patients at the study centre rather than by a power calculation.

6.3.2.3 Haematological evaluation

The main objective of the study was to look for evidence of an antisickling effect *in vivo*. The following tests were performed to look for such an effect:

- ^{51}Cr erythrocyte survival;
- glutaraldehyde-fixed blood film counts as an assessment of % irreversibly sickled cells (ISC's)
- haemoglobin, reticulocytes, bilirubin, plasma lactate dehydrogenase (LDH) levels as indices of the rate of haemolysis.

6.3.2.4 Exercise tests

The previous study demonstrated that the effect of tucaresol on the cardiovascular system is more apparent under moderate exercise than at rest. Patients with sickle cell disease already have an elevated cardiac output at rest to maintain tissue oxygen delivery (Pianosi *et al*, 1991). Under conditions of increased oxygen demand such as during exercise, the necessary increase in oxygen delivery is met partially by further increases in cardiac output but also by an increase in arterio-venous oxygen difference (Pianosi *et al*, 1991). An increase in the latter is potentially deleterious in patients with sickle cell disease as it reported that exercise can lead to an increased risk of sickling and sudden death (reviewed by Martin *et al*, 1989). Hence this study incorporated mild - moderate exercise titrated to produce a peak heart rate of 140 bpm with a Borg Scale as a measure of perceived exertion. Cardiac output was assessed by echocardiography.

6.3.2.5 Safety considerations

The initial cohort was planned to include 4-6 patients depending on availability. Provided that there were no unacceptable adverse experiences, further similar cohorts were to be entered into the study at intervals of not less than 2 weeks. During the treatment phase, ECG was constantly monitored using an intelligent alarm system (HP78560A) connected to bedside monitors (HP78352A) or via telemetry (HP78101A).

6.4 Subjects, Protocol and Methods

6.4.1 Subjects

Between 18-24 non-smoking stable patients with sickle cell disease of either sex, aged 18-50, were to be recruited from the Department of Haematology, King's College Hospital. All patients underwent a screening medical examination which comprised history, physical examination, 12-lead ECG, full blood count including reticulocyte count, haemoglobin electrophoresis and plasma biochemistry. Patients were required to have HbSS phenotype, and be stable as defined by no crisis in the last 7 days and have a haemoglobin within 1 gm/100 ml of their usual value from the departmental records. Women were required to be using an adequate and reliable form of contraception throughout the study. Exclusion criteria were :

- HbA levels $>10\%$;
- blood transfusion within the last 3 months;
- significant hepatic disease defined by AST, ALT or ALP greater than twice the upper limit of normal;

- significant renal disease defined by plasma creatinine $>150\ \mu M$;
- significant cardiac failure as evidenced by a previous episode of pulmonary oedema;
- the presence of another significant medical condition other than the complications of sickle cell disease;
- sufficiently poor peripheral veins to make repeated venepuncture difficult;
- likely poor compliance with the protocol (in the opinion of the investigator).

6.4.2 Protocol

The study protocol was approved by the Camberwell Health Authority Ethics Committee.

6.4.3 Drug Administration and Dosages

6.4.3.1 Loading dose

In the first volunteer study, peak %MOD ranged between 19-26% after a single dose of 3600mg. Allowing for some drug distribution and elimination it was estimated that with a daily dose of 1200 mg the target range of 20-25 %MOD for this study would probably be reached after 3-5 days. However, because of the relationship between peak %MOD and body weight, the loading dose was reduced to 800 mg daily for patients less than 70 kg. The loading dose was administered as 400 mg tablets. The following adjustments to the loading dose were planned to minimise under- or over-shoot.

In patients receiving 1200 mg/day:

- if %MOD after 2 doses was 17 or 18% subsequent loading was to be reduced to 800 mg per day;
- if %MOD after 2 doses was 19% subsequent loading was to be reduced to 400 mg per day;
- if %MOD after 3 doses was 19% subsequent loading was reduced to 800 mg per day.

In patients receiving 800 mg/day:

- if %MOD after 2 doses was 17 or 18% subsequent loading was to be reduced to 400 mg per day;
- if %MOD after 3 doses was 19% subsequent loading was to be reduced to 400 mg per day;
- if %MOD after 3 doses was 13% or less subsequent loading was to be increased to 1200 mg per day.

6.4.3.2 Maintenance dose

Once %MOD was greater than or equal to 20% following the loading dose, patients were changed to the maintenance dose. The daily maintenance dose (Dose) was calculated from the following equation :

$$Dose = C_e \cdot CL_e$$

Equ 6.1

where C_e is the erythrocyte concentration estimated to produce 20-25 %MOD and CL_e is the apparent clearance from erythrocytes. C_e was taken as the mean

erythrocyte concentration following 3600 mg in the first volunteer study which produced a mean peak %MOD of 22.8% (Table 4.2). CL_e was calculated from the same group as :

$$CL_e = \frac{Dose}{AUC_e}$$

Equ 6.2

where the dose was 3600 mg and AUC_e was the mean $AUC_{0-\infty}$ in erythrocytes. Using values of C_e of 1.035 mg/ml and AUC_e of 280 h.mg/ml, the estimated daily maintenance dose was 320 mg/day, which was rounded to 300 mg/day because of the unit doses available.

6.4.3.3 Blinding

In order to maintain blinding, patients in both active and placebo groups received the same number of tablets. Patients received three tablets daily during the loading phase being an appropriate mixture of 400 mg tucaresol tablets or matching placebo. Patients on active drug were transferred to the maintenance dose once >20 %MOD had been achieved; patients on placebo were randomly allocated to the maintenance dose level between 3 and 5 days from the start of dosing. During the maintenance phase all patients received 5 tablets daily being an appropriate mixture of 100 mg tucaresol tablets or matching placebo.

6.4.3.4 Drug administration

All doses were taken with 250 ml water approximately 3 h after breakfast.

6.4.4 Schedule

6.4.4.1 Screening phase

This consisted of a single outpatient visit within 1 month of the start of the treatment phase.

6.4.4.2 Baseline phase

In this phase, the erythrocyte survival half-life was assessed. Patients visited the Investigation Unit to familiarise themselves with it and performed a supine exercise test at work rates of 40, 80 and 120 W to determine a work rate which produced a heart rate of approximately 140 bpm. The baseline phase took place within 2 weeks of the start of the treatment phase.

6.4.4.3. Treatment phase

Patients were required to abstain from alcohol for 24 h before admission to the Study Unit. They were brought to the Study Unit on the morning of day 0. An exercise test was performed in the afternoon of day 0. On days 1-10, a blood sample was taken at approximately 7 am, breakfast was taken subsequently and the dose of study drug at 10 am. An exercise test was scheduled at 3 pm on days 2, 4, 6, 8, and 10.

Day	Time	Procedure
0	1000	Admission to Unit

	1500	Exercise test
1-10	0700	Blood sample*
	0715	Breakfast
	0900	Heart rate, blood pressure, adverse events
	1000	Dose
	1200	Lunch
	1500	Exercise test (days 2, 4, 6, 8 and 10)
		Evening meal as desired
10	1700	Discharge from Unit.

* Blood samples were taken for the following assessments:

- %MOD, plasma and whole blood tucaresol concentrations, days 1-10
- Full blood count and reticulocytes, biochemistry, ISC, days 0, 5 and 10
- HbF days 0 and 10

6.4.4.4 Follow-up phase

Patients were seen at weekly intervals for four visits in the Haematology Outpatient Clinic following discharge from the Unit. Adverse events were assessed and blood taken for plasma and whole blood tucaresol concentration, full blood count and reticulocytes, ISC and %MOD until the latter was $< 5\%$.

6.4.5 Clinical measurements and procedures

The exercise tests were to be performed as described in the last chapter, including a Borg scale to assess perceived level of exertion. As the BoMed NCCOM 3 bioimpedance cardiography method used in the previous study did not appear to perform well under exercise, attempts were made to measure cardiac output during exercise by echocardiography. Using this approach, stroke volume could be calculated from the difference in end-diastolic and end-systolic volumes (Kronik *et al*, 1979). Multiplying stroke volume by heart rate produces an estimate of cardiac output.

6.4.6 Laboratory methods

Plasma and haemolysate tucaresol concentration and %MOD were measured as described previously, except that, to ensure complete extraction of tucaresol from the haemolysate, the samples were left for 15 min after acidification before toluene extraction. Full blood counts and reticulocytes were performed using standard laboratory methods as was clinical biochemistry. Haemoglobin A, S and F were measured by electrophoresis (Huisman, 1986). The erythrocyte survival was assessed using a standard method (International Committee for Standardization in Haematology, 1980). On two occasions, twenty ml blood samples were taken from the patient and labelled *ex vivo* with ^{51}Cr, and then reinfused into the patient. The first occasion was during the screening phase and the second at the end of the dosing phase. Samples for whole blood radioactivity were taken during the three week period between the first infusion and the end of the dosing period, and for four weeks after the second infusion. After correction of the counts for

radiochemical decay of ^{51}Cr, the half-life of blood radioactivity is a measure of the erythroctye survival half-life.

6.4.7 Data analysis

The protocol stated that differences between treatment groups in :

- indices of haemolysis (Hb, reticulocytes, bilirubin, ISC's);
- cardiac output, heart rate and Borg scales during exercise

were to be compared by analysis of variance. However, becasuse of the small number of patients completing the study and the modifications to the dosing regimen, no formal statistical analysis was performed.

Pharmacokinetic analysis was performed as described in Chapter 3.

6.5 **Results**

6.5.1 Subjects

Twelve patients completed the study, in 4 cohorts of three subjects each containing one subject on placebo. The size of the cohorts and overall number were less than intended due to problems with patient recruitment. Their demographics are listed in Table 6.1. Nine were men and three were women. The mean (SD) age was 26.9 (6.8) y and weight 65.1 (8.3) kg. All patients except two had HbSS phenotype; Subject 2 had HbSßthal phenotype and Subject 9 had HbSC phenotype. All patients were anaemic with Hb levels at screen ranging from 6.5-11.0 g/dl. All patients had multiple hospital admissions for management of sickle cell crises.

6.5.2. Protocol compliance and modifications

The protocol underwent several major revisions during the study. These are discussed below.

6.5.2.1 Omission of exercise tests

Many patients had difficulty performing the exercise tests, usually due to pre-existing chronic bone pain, and some were unwilling to continue the tests throughout the study. It was also difficult to obtain reliable stroke volume assessments during exercise because of the subjects' hyperdynamic circulation with readings often only being available more than a minute after the end of the test. As it was unlikely that the tests could be performed and analysed consistently throughout the study, and because these data were not critical to the study, the tests were performed less frequently than scheduled and in some subjects cardiac output measurements were only made at rest and in others the tests were omitted entirely.

6.5.2.2 Omission of the loading dose

The loading dose was omitted after the first cohort had completed the treatment phase. This was for two reasons:

- Subject 2 developed significant adverse experiences (section 6.5.6)
- Subject 3 developed a large rise in haemoglobin level (section 6.5.7)

6.5.2.3 Reduction in maintenance dose

In addition to the omission of the loading dose after the first cohort and as a result of the development of adverse experiences in two subjects in the third cohort, the maintenance dose was reduced from 300 mg/day to 50 mg/day to explore whether there was a dose which could be well tolerated. If 50 mg/day was well tolerated by 9 patients the dose was to be increased to 100 mg/day; however not enough patients were recruited to proceed to this phase and only two subjects received 50 mg/day before the study was closed.

6.5.3 Doses administered

A summary of the doses administered is in Table 6.2.

6.5.4 Pharmacokinetics

Individual plasma concentration-time profiles are plotted in Figure 6.1 on arithmetic axes and in Figure 6.2 on semilogarithmic axes. Individual plasma pharmacokinetic parameters are listed in Table 6.3. Mean values have not been calculated because of the different doses received by patients in different cohorts and the trends for many pharmacokinetic parameters of tucaresol to be dose- and concentration-dependent. Plasma C_{max} values in Subjects 1-9 ranged from 39.8 to 77.2 μg/ml. Although C_{max} values were higher in the the two men who received the loading dose compared to those who had only received the maintenance dose, the C_{max} values in the two women who received only the maintenance dose were similar to those in the two men who received the loading dose. Plasma half-life ranged from 92.6 h to 215.6 h

with the values from the two subjects who received 50 mg/day being among the lowest (92.6 and 99.2 h).

Individual erythrocyte concentration-time profiles are plotted in Figure 6.3 on arithmetic axes and in Figure 6.4 on semilogarithmic axes. In most profiles there was evidence of the rate of decline of concentrations slowing after 720 h. Pharmacokinetic parameters for tucaresol concentrations in erythrocytes are also listed in Table 6.3. C_{max} values in Subjects 1-9 ranged from 453.0 to 944.8 μg/ml. The two highest values were in the two subjects who received the loading dose. Tucaresol half-life from erythrocytes ranged from 54.4 to 115.7 h in all subjects.

6.5.5 Haemoglobin modification - %MOD

Individual %MOD values are listed in Tables 6.4 and individual %MOD-time profiles are displayed in Figure 6.5. Haemoglobin modification was not detected in any patient on placebo or the patients receiving the low dose of 50 mg/day. In the two subjects receiving tucaresol from the first cohort, peak %MOD values were 23 and 24% on day 5. By the end of the maintenance phase (day 11), values had declined to 13 and 21% respectively. Modification was not detected by day 23. In the second and third cohorts peak %MOD values ranged between 10 and 18%, and occurred on either day 11 or 12. The two women had higher values than the men. Modification was not detected after day 19.

6.5.6 Haematology

6.5.6.1 Blood Counts

Individual subject's Hb levels are listed in Table 6.5 and plotted in Figure 6.6 as percent of the individual baseline values on day 1. There were no trends for changes in the subjects who received placebo. In the two subjects who received tucaresol with a loading dose, rises in Hb were seen. In subject 2, who has HbSßthal phenotype, Hb increased from 8.9 mg/dl on day 1 to 10.9 mg/dl on days 11 and 16, with a gradual decline to 10.4 mg/dl at the end of the study. In subject 3, the increase was greater, from 9.5 mg/dl on day 1 to a peak of 13.2 mg/dl on day 16, with a gradual decrease to 10.2 mg/dl at the end of the study. In the three subjects with HbSS who received the maintenance dose but no loading dose, rises in Hb also occurred, ranging from 1.8 - 2.2 mg/dl, occurring between day 16 and the 2nd follow-up visit. In these three subjects values had largely returned to baseline by the end of the study. The other patient who received the maintenance dose but no loading dose had HbSC phenotype and had the highest Hb at the beginning of the study, and there was no trend for change in Hb level during the study. There were no trends for changes in Hb levels in the subjects who received the low dose of tucaresol.

6.5.6.2 Reticulocyte Count

Individual reticulocyte data are listed in Table 6.6 There were no trends for changes in reticulocyte counts in any group.

6.5.6.3 Haemoglobin Electrophoresis

Individual subject's %HbF values are listed in Table 6.7. In two of the subjects who received only the maintenance dose of tucaresol there were large falls in HbF. Subject 4 had 6.2% HbF on day 1 and 1.5% at the end of the study; Subject 6 had values of 9.7% and 2.0% at the same times. There were no clear trends in any of the other subjects receiving tucaresol but their baseline values were much lower than Subjects 4 and 6. There was a small fall in the placebo patient with a high baseline value; Subject 1 had 10.2% HbF on day 1 and 8.8% at the end of the study. Patients in the low dose group had HbF determinations only at screen and hence there is no information about changes during the study.

6.5.6.4 Irreversibly sickled cell count - ISC

Individual ISC counts (as % of total) are listed in Table 6.8 and plotted in Figure 6.7. There were no trends for changes in any of the patients who received placebo but large falls were seen in all patients who received tucaresol, except for the 50 mg/kg group. The maximum percent decreases from day 1 were similar in the groups with and without a loading dose, and ranged from 40-71%, and occurred either on day 11 or the week 1 follow-up visit. Values had largely returned to baseline by the end of the study.

6.5.6.5. ^{51}Cr Erythrocyte Half-life

Erythrocyte half-life tests were only performed in cohorts 2 and 3. They were omitted from the first cohort because the required approval from the Administration of Radioactive Substances Advisory Committee had not been granted when the study

was due to start. The tests were omitted from the subjects receiving the low dose of 50 mg/day because it was thought unlikely that a significant change would occur due to lack of effect on %MOD. Individual values of ^{51}Cr erythrocyte half-life are listed in Table 6.9 and the data (after adjustment for the radiochemical decay of ^{51}Cr) on which half-lives were determined are shown graphically with the period of dosing in Figures 6.8 - 6.13. Half-lives were calculated by log-linear regression over the whole sampling period. In subject 8 the post-treatment half-life was longer than pre-treatment but in all other subjects pre- and post-treatment values were similar.

6.5.7 Adverse experiences

The most frequently reported adverse experience was pain in the limbs or trunk. Adverse experiences other than pain in the limbs or trunk are listed in Table 6.10. Few of these adverse experiences were reported in subjects on placebo. After limb or trunk pain, headache was the most commonly reported adverse experience reported by one subject (Subject 1) on placebo and five subjects on tucaresol (Subjects 2, 3, 4, 8 and 9). Other adverse experiences are outlined below.

Subject 1 reported a heat rash on the trunk and arms with onset day 6 and which lasted three days. He also reported swelling of the left forearm on day 36 which came on after weight training.

Subject 2 (tucaresol group with loading dose) developed a high fever (to 39.4°C) on day 7. Tucaresol administration was withheld from day 8 onwards. On day 8 the patient complained of tenderness in the neck and multiple small tender lymph nodes were detected on examination. The patient was observed over the next few days but as there was no apparent improvement, prednisolone 25 mg stat followed by 15 mg bd was started in the afternoon of day 11. There was substantial improvement by the next morning with reduction of temperature and less tenderness of the nodes.

On day 26, subject 3 (tucaresol group with loading dose) reported tenderness in the right axilla with onset the previous day. On examination, no definite node was detected, but a small non-tender node was palpated in the left axilla. A few small nodes were felt in the inguinal regions but these were considered to be normal. No other nodes were palpable but splenomegaly was readily detected and confirmed by ultrasound. This had not been detected at the screening medical examination. The splenomegaly was still present at the final medical examination on day 44.

Subject 4 (tucaresol group without loading dose) reported a rash on the left forearm in the evening on day 3. There were no other symptoms at the time. The rash had disappeared by the following morning when he was examined by a physician who reported no abnormalities. On day 7 the patient again reported a rash on the left forearm which was described as itchy and papular. The duration was 5.5 h.

Subject 5 (placebo) reported indigestion coming on 9.75 h after the first dose of tablets, for which an antacid was administered.

Subject 6 (tucaresol group without loading dose) was admitted to hospital on day 16 because of headache and vomiting of three days duration. He was managed with iv fluids and oral analgesics. Following admission his symptoms largely settled within a day and he was discharged on day 19. He also reported mild abdominal pain on day 39.

Subject 7 (placebo) reported no adverse experiences.

In the evening of day 9, Subject 8 (tucaresol group without loading dose) reported headache and a sore throat. On examination, there was thought to be mild inflammation of the pharynx but no lymph nodes were palpable. The temperature was 37.2 °C. At a follow-up visit on day 14, she reported that the tenderness had continued without pain on swallowing but that the neck was tender on the outside. On examination, a number of small tender nodes were palpated on both sides of the neck. The temperature was 37.5°C. On day 18 the symptoms were less but a few tender nodes persisted. The temperature was 38°C; there was no splenomegaly. No specific therapy was given.

Subject 9 (tucaresol group without loading dose) reported abdominal cramp on days 3 and 5, 17 and 20 min respectively after dosing. In the evening of day 11, she

developed a fever accompanied by rigors and tenderness on both sides of the neck. When examined the following morning (day 12), the temperature was 39.0 °C. There was tenderness on both sides of the neck but no nodes were definitely palpated and there was no splenomegaly. The was no evidence of pharyngitis. The patient was admitted to hospital because there was concern that the fever might increase the likelihood of clinical sickling. On day 12 prednisolone 25 mg stat and 15 mg bd was commenced. At 1200 h on day 13 the patient was noted to be afebrile and was clinically improving. On examination on day 18 there was less tenderness of the neck and the temperature was 37.2°C. On examination on day 22 there were no signs in the neck and the temperature was 37.5°C.

Subject 10 (tucaresol group - low dose) reported a mild sore throat with onset on day 18 which lasted for 3 days. There are no details of physical examination at the time. Mildly elevated but asymptomatic temperature was recorded with the highest reading of 37.8° being on day 16.

Subject 11 (tucaresol group - low dose) reported no adverse experiences.

Subject 12 (placebo) reported no adverse experiences but a temperature of 37.7°C was noted on day 11.

6.5.8 Biochemistry

Individual values of LDH and total bilirubin are in Tables 6.11 and 6.12 and plotted in Figures 6.14 and 6.15 respectively as percent of the individual baseline values on day 1. LDH values decreased in all subjects who received tucaresol except the low dose pair, with slightly greater falls in those who received the loading dose compared to those who did not. There was no trend for a fall in the placebo patients. Bilirubin values fell in all subjects who received tucaresol except the low dose pair, with similar falls in those with and without the loading dose. There were no trends for changes in the placebo group.

6.5.9 Exercise tests

Due to the intermittent recordings of stroke volume, heart rate and cardiac output at rest and under exercise these are not tabulated. There were no trends for treatment-related differences.

6.6 **Discussion**

As mentioned in Chapter 3, initial dosing of valeresol in patients with sickle cell disease compared to normals because it was thought that the pharmacokinetics of valeresol might be different between the two groups because of the effect of anaemia. It was found however that the doses required to produce the same %MOD were similar in the two groups. In order to compare the pharmacokinetics of tucaresol between patients and normals, individual values of plasma clearance from all three human studies have been plotted against individual values of plasma C_{max} in Figure 6.16 and individual values of plasma half-life against individual values of

plasma C_{max} in Figure 6.17. Similarly, individual values of tucaresol clearance from erythrocytes have been plotted against individual values of tucaresol C_{max} in erythroctyes in Figure 6.18. These plots are more useful to compare pharmacokinetics between the studies and subject groups than mean pharmacokinetic parameters, because of the trends for dose- and concentration- dependent kinetics demonstrated in the first volunteer study and the different dosing regimens within and between the studies. It is clear from Figure 6.16 that tucaresol plasma clearance is similar in patients and volunteers. Figure 6.17 shows that the trend for increasing plasma half-life with increasing plasma concentration is similar in patients and volunteers. Figure 6.18 shows that clearance of tucaresol from erythrocytes is lower at higher concentrations, but in the same concentration range, the values are similar for patients and volunteers. As the relationship between erythrocyte tucaresol concentration and %MOD is similar in patients and volunteers (see Figure 6.19), it is unlikely that tucaresol dosing, based on pharmacokinetics and %MOD data in healthy volunteers, will need to be adjusted for anaemia in patients, as is also the case for valeresol. In the four subjects who received a fixed dose of 300 mg/day, lower clearance values and higher peak %MOD occurred in the two women compared to the men. The higher %MOD and lower clearance cannot be attributed to reduced body weight as body weight was similar between the women and the men. In all animal species studied, tucaresol had a lower clearance and longer half-life in females compared to males. Although the amount of data is limited it is possible that women also may have a reduced clearance compared to men.

A major objective of this study was to search for evidence of an antihaemolytic effect of tucaresol. In the two patients who received the loading dose of tucaresol, and hence maintained peak %MOD in the 20-25% target range, there were rises in Hb and decreases in LDH and bilirubin consistent with an antihaemolytic effect. The rises in Hb are unlikely to be due to stimulation of erythropoietin production, since there was no concomitant increase in reticulocyte count and since healthy volunteers who achieved a significantly greater peak %MOD also had no reticulocytosis or rise in Hb (see Chapter 5). Although Subjects 2 and 3 attained similar peak %MOD values (23% and 24% respectively), this level was not maintained during the remainder of the dosing phase in Subject 2 and had declined to 13% on the last day of the residential phase (day 11). In Subject 3 however, %MOD was 21% on day 11. The maintenance of higher %MOD values for longer in Subject 3 may have been a major factor in the large increase in Hb levels of 3.7 gm/dl. Such a rapid rise in Hb levels may be clinically undesirable due to a possible increase in the probability of clinical sickling and this was one factor in the decision to omit the loading dose for subsequent cohorts. The three subjects of HbSS phenotype who received the maintenance dose without the loading dose (Subjects 4, 6 and 8) also had rises in Hb and falls in LDH and bilirubin suggestive of an antihaemolytic effect but these in general were smaller than in the subjects who also received the loading dose. Subject 9 had the highest baseline Hb, consistent with HbSC disease and presumably had the lowest rate of haemolysis. This may be why changes in Hb, LDH and bilirubin were not as clear in this subject compared to the others. In the two subjects who received low dose tucaresol, no Hb modification

was detected and there were no trends indicative of inhibition of haemolysis. Thus there seems to be a strong correlation between the extent of haemoglobin modification and inhibition of haemolysis with effects seen at >10 %MOD. The peak effects tended to occur either at the end of the dosing phase or within one week of the end of dosing. This slight delay presumably reflects the balance between the rates of erythrocyte production and destruction.

Despite clear haematological and biochemical evidence of a reduction in haemolysis in all but the low dose group, in only one subject (no 8) was there a clear change in erythrocyte half-life. The lack of consistent changes in erythrocyte half-life may have been due to the long sampling schedules required for determination of erythrocyte survival and because the period of dosing actually spanned the end of the "pre-treatment" first measurement and only the beginning of the "post-treatment" second measurement, as shown in Figures 6.8-13. Close inspection of all the plots shows a trend for a longer half-life towards the end on the first assessment period, coincident with the period of dosing. Thus the long sampling period required for accurate determination of erythrocyte survival may make it insensitive to the effects of tucaresol when given over a short period as in this study, and other markers of erythrocyte turnover (haemolysis) may be more sensitive.

In all subjects who received tucaresol except the low dose group there were substantial falls in ISC counts. This is further evidence that the antihaemolytic effects of tucaresol are due to an antisickling effect. However, there is a notoriously

poor correlation between ISC count and clinical sickling (McCurdy and Sherman, 1978; Serjeant *et al*, 1978; Serjeant 1992), and although the haematological and biochemical data are encouraging, it is not possible to confidently predict that this extent of Hb modification with tucaresol could prevent the clinical manifestations of the disease. The dosing period in this study was too short to see an effect on prophylaxis of crisis. Patients in both active and placebo groups reported high rates of limb and trunk pain but often these were manifestations of long-standing pain and hence it is not appropriate to compare reports of pain in the two groups as a clinical index of sickling. Only a longer-term placebo-controlled study would be able to determine whether this level of Hb modification might be clinically beneficial.

Another objective of the study was to examine the tolerability of tucaresol, particularly in the light of the adverse experiences reported in the second volunteer study. Three patients in this study developed fever and probable tender cervical lymphadenopathy. In two patients the symptoms were sufficiently uncomfortable to require intervention and corticosteroids produced rapid major improvement. In the third patient the symptoms were much milder and reported largely retrospectively: no treatment was required. These symptoms and the timing from onset of dosing were very similar between the second volunteer study and this study with the exception of rash in the patients. However, as the rash was macular in the healthy volunteer study it would have been difficult to observe on the dark skins of the patients in this study. The consistency of the timing from start of dosing of 7-10 days is remarkable given the very different dosing regimens in the healthy

volunteers who received three large doses at 48 hr intervals and the patients who received a daily dose sometimes with a load for the first few days. The proportion of subjects affected in the two studies is also similar; 3/8 subjects in the healthy volunteers and 3/6 in the patients excluding the low dose group or 3/8 including the low dose group. The similarities in timing and frequency, combined with the large differences in peak %MOD suggest that multiple dosing with tucaresol produces these adverse experiences independent of a mechanism related to %MOD or dosing regimen. Subsequent laboratory investigations indicated a possible mechanism for the adverse experiences and this will be discussed in the next chapter.

6.7 Conclusions

- The pharmacokinetics of tucaresol are similar in patients and healthy volunteers.

- Plasma clearance of tucaresol may be lower in women than in men.

- The relationship between erythrocyte tucaresol concentrations and %MOD is similar in patients and healthy volunteers.

- Haemoglobin modification by tucaresol of between 10-24% in stable patients with sickle cell disease has an anti-haemolytic effect evidenced by increased haemoglobin, reduced ISC count and reduced bilirubin.

- The administration of daily doses of tucaresol, with or without a loading dose, is accompanied in some subjects by fever and tender cervical lymphadenopathy which responds well to prednisolone.

- Further clinical development of tucaresol will depend on whether a dosing strategy can be developed which will avoid or reduce the risk of drug allergy.

TABLES

Table 6.1 Patient demographics, haematology and treatment allocation

Patient Number	Sex	Age y	Weight kg	Hb* g/100 ml	Hb phenotype	Treatment
1	M	22	64	8.3	SS	Placebo
2	M	27	65	9.5	SßThal	Tucaresol
3	M	17	73	10.6	SS	Tucaresol
4	M	29	72	8.6	SS	Tucaresol
5	M	28	66	8.9	SS	Placebo
6	M	28	57	8.8	SS	Tucaresol
7	M	24	55	7.5	SS	Placebo
8	F	32	62	8.3	SS	Tucaresol
9	F	39	83	11.0	SC	Tucaresol
10	F	37	69.5	9.4	SS	Tucaresol
11	M	20	60	8.8	SS	Tucaresol
12	M	20	55	6.5	SS	Placebo
Mean	-	26.9	65.1	8.9	-	-
SD	-	6.8	8.3	1.2	-	-

* at screen

Table 6.2 Dosing summary

Patient Number	Loading Dose (mg/day)	Number of days of Loading Dose	Total Loading Dose (mg)	Maintenance Dose (mg/day)	Number of days of Maintenance Dose	Total Maintenance Dose (mg)	Total Dose (mg)
2	800	4	3200	200	4	800	4000
3	1200	4	4800	300	6	1800	6600
4	-	-	-	300	10	3000	3000
6	-	-	-	300	10	3000	3000
8	-	-	-	300	10	3000	3000
9	-	-	-	300	10	3000	3000
10	-	-	-	50	10	500	500
11	-	-	-	50	10	500	500

Table 6.3 Summary of pharmacokinetic and %MOD data

Subject No	Dose (mg)	Plasma						Erythrocyte				%MOD		
		C_{max} $\mu g\ ml^{-1}$	t_{max} h	$AUC_{0-\infty}$ $h.\mu g\ ml^{-1}$	$t_{1/2}$ h	CL/F $ml\ min^{-1}$	V_z/F l	C_{max} $\mu g\ ml^{-1}$	t_{max} h	$AUC_{0-\infty}$ $h.\mu g\ ml^{-1}$	$t_{1/2}$ h	Max. %MOD	t_{max} h	$t_{1/2}$ h
2	4057	61.3	91.9	20771	155.4	3.26	43.8	795.4	91.9	219220	71.7	23	91.9	137.5
3	6690	60.5	91.8	25225	215.6	4.42	82.5	944.8	139.8	320619	115.7	24	91.8	107.9
4	3023	39.8	189.4	14286	126.9	3.54	38.9	453.0	237.4	108379	90.2	10	261.6	67.7
6	3023	43.9	236.8	12972	94.6	3.88	31.8	608.4	236.8	147113	54.4	14	236.8	116.0
8	3023	77.2	262.3	25767	133.4	1.96	22.6	661.8	238.1	211428	75.4	15	214.2	178.8
9	3023	60.2	262.5	21596	135.1	2.33	27.3	750.4	238.6	219136	71.0	18	238.6	139.3
10	504	9.7	123.1	2973	92.6	2.83	22.7	32.7	123.1	8553	60.6	ND	-	-
11	504	9.5	262.8	3056	99.2	2.75	23.6	19.7	262.8	4667	67.1	ND	-	-

ND = not detected

Table 6.4 %MOD data

Study Time	With load		Without load			
	Sub 2	Sub 3	Sub 4	Sub 6	Sub 8	Sub 9
Day 1	<5	<5	<5	<5	<5	<5
Day 2	5	7	-	-	-	-
Day 3	12	12	-	-	-	-
Day 4	18	19	-	-	-	-
Day 5	23	24	5	7	8	9
Day 6	20	22	-	-	-	-
Day 7	21	22	8	9	11	13
Day 8	19	21	9	11	12	13
Day 9	19	23	8	12	13	16
Day 10	15	23	9	12	15	15
Day 11	13	21	9	14	15	18
Day 12	-	-	10	11	13	14
Day 14	-	-	5	8	9	11
Day 15	-	-	5	7	8	12
Day 16	7	12	-	-	-	-
Day 18	-	-	<5	<5	8	9
Day 19	5	5	-	-	-	-
Day 22	-	-	-	-	<5	<5
Day 23	<5	<5	-	-	-	-

Table 6.5 Haematology data - Haemoglobin (mg/dl)

Study Time-	Placebo group				Tucaresol group							
					With load		Without load				Low dose	
	Sub 1	Sub 5	Sub 7	Sub 12	Sub 2	Sub 3	Sub 4	Sub 6	Sub 8	Sub 9	Sub 10	Sub 11
Screen	8.3	8.9	7.5	6.5	9.5	10.6	8.6	8.8	8.3	11.0	9.4	8.8
Day 1	8.3	8.6	6.4	6.9	8.9	9.5	8.4	7.7	7.9	10.7	9.2	9.0
Day 5	8.6	8.6	7.1	6.2	9.7	11.2	9.1	8.9	9.0	11.5	9.0	8.7
Day 11	8.9	9.0	6.1	6.1	10.9	11.9	9.6	9.7	9.4	-	-	8.5
Day 16	8.9	-	-	-	10.9	13.2	10.7	9.4	-	-	-	-
Wk 1	8.8	9.2	7.4	6.2	10.9	13.1	9.7	8.4	10.1	11.2	9.3	8.1
Wk 2	9.4	9.2	7.5	5.9	10.8	11.4	8.8	9.5	8.9	11.6	9.2	-
Wk 3	9.5	-	-	-	10.4	10.1	8.3	8.6	-	10.5	9.1	8.9
Wk 4	9.1	9.7	7	6.5	10.4	10.2	8.3	8.2	7.5	9.8	-	8.8

Table 6.6 Haematology data - Reticulocyte count (%)

Study Time	Placebo group				Tucaresol group							
					With load		Without load				Low dose	
	Sub 1	Sub 5	Sub 7	Sub 12	Sub 2	Sub 3	Sub 4	Sub 6	Sub 8	Sub 9	Sub 10	Sub 11
Screen	-	7.2	6.8	4.8	1.2	-	7.8	6.4	3.5	2.2	4.6	3.2
Day 1	2.0	5.3	10.8	10.6	3.2	2.3	6.1	4.6	4.9	2.7	7.3	6.0
Day 5	3.8	6.3	6.9	4.4	6.2	4.0	5.5	4.1	3.7	2.3	3.2	5.2
Day 11	5.3	5.3	6.0	4.5	2.7	3.2	7.4	3.9	4.5	-	-	5.9
Day 16	4.4	-	-	-	3.3	1.1	5.8	3.9	-	-	-	-
Wk 1	5.0	5.2	7.4	6.0	3.8	2.6	3.1	4.0	1.3	2.4	5.7	3.5
Wk 2	2.7	5.3	5.2	4.4	2.6	2.4	7.0	3.8	2.9	1.9	5.4	-
Wk 3	4.8	-	-	-	2.7	2.8	6.0	3.6	-	-	8.1	3.7
Wk 4	1.2	4.3	-	6.1	1.8	4.0	6.4	6.0	4.3	3.0	-	3.8

Table 6.7 Haematology data - Haemoglobin F (%)

Study Time	Placebo group				Tucaresol group							
					With load		Without load				Low dose	
	Sub 1	Sub 5	Sub 7	Sub 12	Sub 2	Sub 3	Sub 4	Sub 6	Sub 8	Sub 9	Sub 10	Sub 11
Screen	9.2	1.1	2.7	4.6	0.3	0.6	6.1	10.1	4.4	1.4	14	4
Day 1	10.2	1.1	2.2	-	0.4	0.6	6.2	9.7	3.9	1.2	-	-
Day 11	8.1	1.3	1.6	-	0.8	0.9	6.1	11.6	2.1	0.3	-	-
Wk 4	8.8	0.5	-	-	0.3	0.6	1.5	2.0	2.8	0.9	-	-

Table 6.8 Haematology data - Irreversibly Sickled Cell Count (%)

Study Time	Placebo group				Tucaresol group							
					With load		Without load				Low dose	
	Sub 1	Sub 5	Sub 7	Sub 12	Sub 2	Sub 3	Sub 4	Sub 6	Sub 8	Sub 9	Sub 10	Sub 11
Day 1	22	19	19	27	21	20	18	22	34	11	27	22
Day 5-7	21	20	17	28	14	14	10	14	17	7	25	20
Day 11-12	18	22	24	26	10	11	9	13	10	6	20	20
Day 15	-	-	-	28	-	-	-	-	-	-	22	25
Day 18-19	20	18	-	-	9	12	14	15	12	10	-	-
Day 22-26	20	21	19	-	12	16	13	21	22	9	25	-
Day 32-33	21	-	-	25	20	17	19	-	-	-	22	-
Day 40-43	18	19	-	-	19	20	19	19	-	-	-	21

Table 6.9 ^{51}Cr erythrocyte half-life values (h)		
Subject no	Pre-treatment	Post-treatment
Placebo group		
1	NP	NP
5	10.3	12.6
7	10.6	11.1
Tucaresol group		
2	NP	NP
3	NP	NP
4	12.2	10.8
6	10.8	11.1
8	9.5	15.5
9	21.1	26.0

NP = test not performed

Table 6.10 Adverse experiences			
Subject No	Study Day	Adverse Experience	Treatment
Placebo subjects			
1	11	Headache	None
	36	Swollen forearm after exercise	None
5	1	Indigestion	Antacid
7	-	None	-
12	11	Fever 37.7 C	None
Tucaresol subjects			
2	3	Headache	Paracetamol
	4	Headache	Paracetamol
	5	Headache	Paracetamol
	7	Headache	Paracetamol
	7	Fever 39.4 C	Paracetamol
	8	Headache	Paracetamol
	10	Headache	Paracetamol
	11	Cervical lymphadenopathy	Prednisolone
3	3	Headache	Paracetamol
	4	Headache	Paracetamol
	4	Tired	None
	9	Headache	None
	11	Neutropaenia	None
	25	Painful R axillary node	None
	26	Splenomegaly	None
4	1	Headache	Paracetamol
	3	Rash on L forearm	None
	4	Headache	Paracetamol
	5	Headache	Paracetamol
	6	Headache	Paracetamol

	7	Headache	Paracetamol
	8	Headache	Paracetamol
	9	Headache	Paracetamol
	11	Abdominal pain	None
6	12	Diarrhoea and vomiting	Admitted to hospital; iv fluids
	14	Feeling weak and hot	None
	39	Abdominal pain	None
8	9	Sore throat	None
	9	Headache	Dihydrocodeine
	10	Tender cervical lymphadenopathy	None
9	0 (Predose)	Migraine	Coproxamol
	2	Headache	Coproxamol
	3	Stomach cramp	Dihydrocodeine
	5	Stomach cramp	Dihydrocodeine
	11	Fever 39.0, neck pain	Admitted to hospital
10	5	Fever 37.4 C	None
	15	Sore throat	None
11	-	None	-

Table 6.11 Biochemistry data - Lactate Dehydrogenase (IU)

Study Time	Placebo group				Tucaresol group							
					With load		Without load				Low dose	
	Sub 1	Sub 5	Sub 7	Sub 12	Sub 2	Sub 3	Sub 4	Sub 6	Sub 8	Sub 9	Sub 10	Sub 11
Screen	944	1263	597	984	746	539	708	873	871	407	888	517
Day 1	659	738	807	1478	835	557	568	894	796	481	852	766
Day 5	630	786	782	1082	776	456	403	778	722	374	754	596
Day 11	658	807	914	1390	681	336	401	697	673	374	812	745
Wk 1	726	-	-	1438	470	312	588	996	537	829	834	815
Wk 2	758	776	761	-	532	270	863	1041	855	399	740	-
Wk 3	692	-	-	1596	796	477	636	915	-	431	757	-
Wk 4	929	710	1031	-	644	640	647	995	828	529	854	895

Table 6.12 Biochemistry data - Bilirubin (μM)

Study Time	Placebo group				Tucaresol group							
					With load		Without load				Low dose	
	Sub 1	Sub 5	Sub 7	Sub 12	Sub 2	Sub 3	Sub 4	Sub 6	Sub 8	Sub 9	Sub 10	Sub 11
Screen	90	70	30	134	19	32	38	52	43	11	34	27
Day 1	78	55	46	175	12	28	69	52	46	13	35	31
Day 5	90	61	30	136	8	16	38	38	43	10	38	34
Day 11	106	54	48	166	15	12	33	34	26	8	-	-
Wk 1	125	-	-	127	15	14	65	50	6	25	41	35
Wk 2	121	76	61	-	17	23	69	40	29	9	41	-
Wk 3	127	-	-	166	8	31	87	52	-	16	38	-
Wk 4	121	70	46	-	18	33	51	28	49	17	36	37

FIGURES

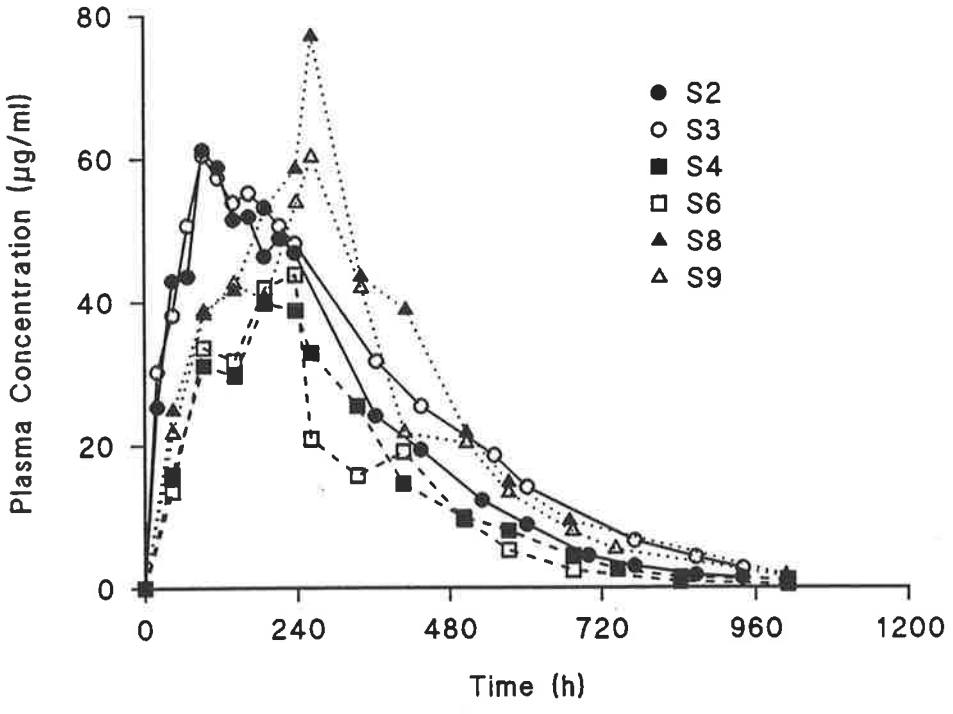


Figure 6.1 Individual tucaresol plasma concentration-time profiles.

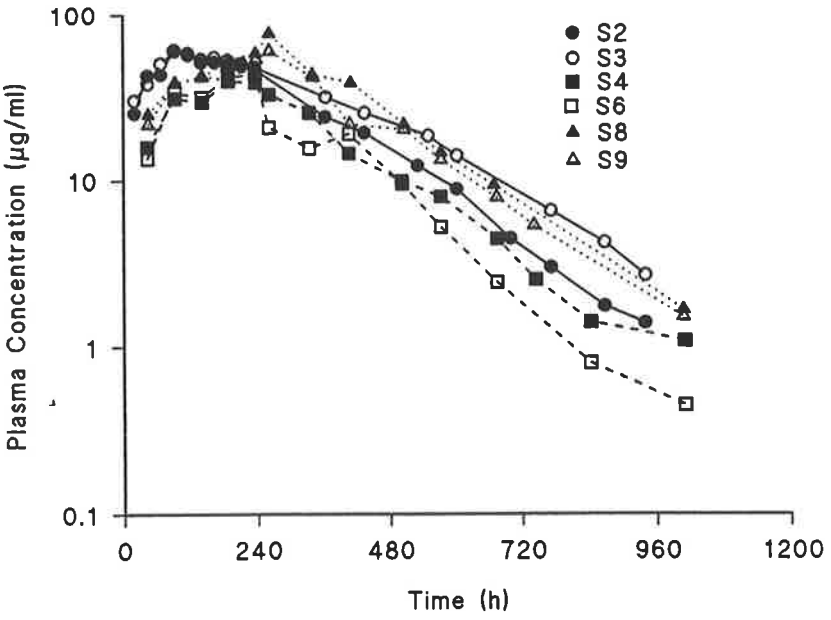


Figure 6.2 Individual tucaresol log-concentration-time profiles.

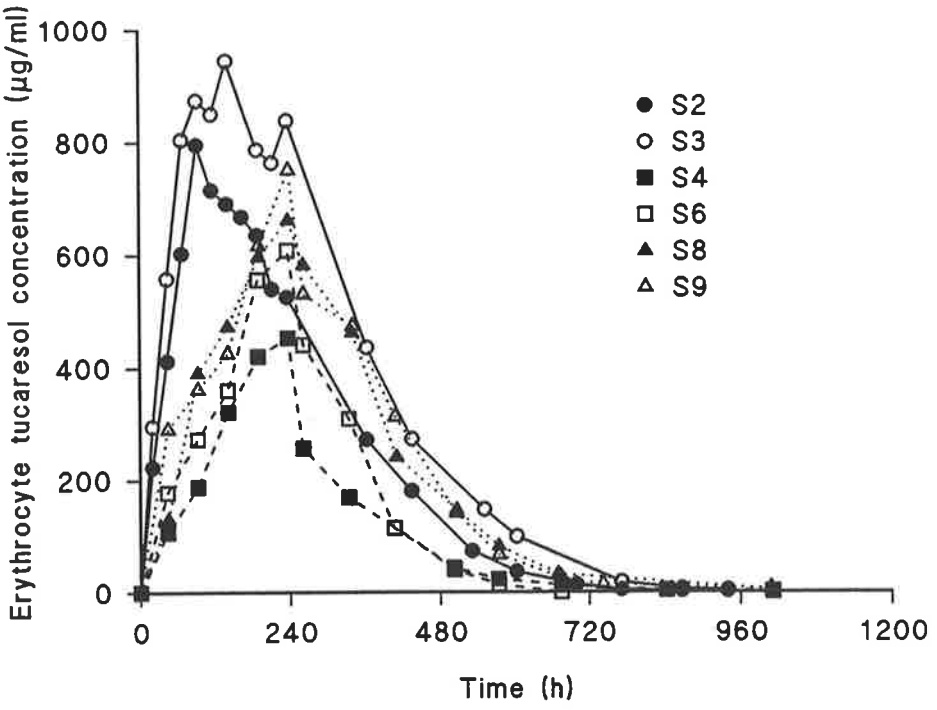


Figure 6.3 Individual erythrocyte tucaresol concentration-time profiles.

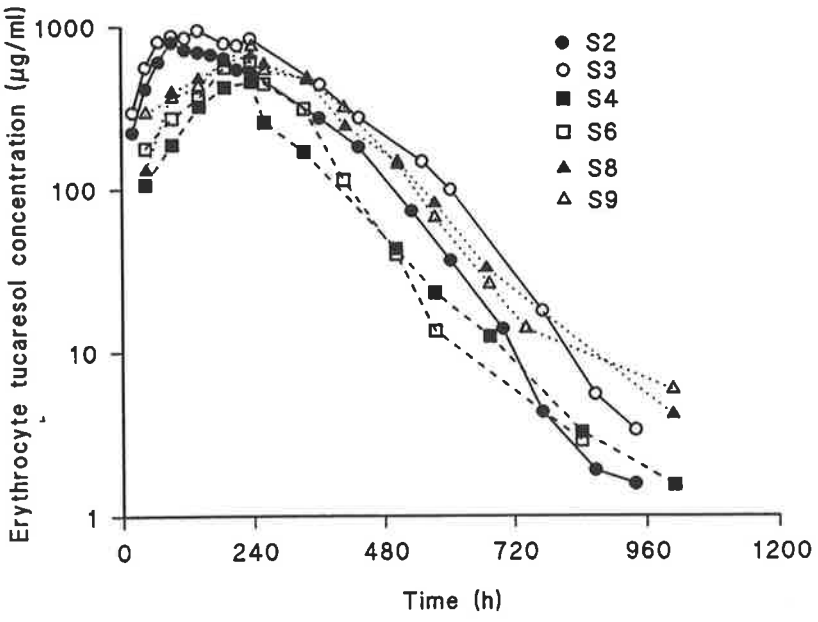


Figure 6.4 Individual erythrocyte tucaresol log concentration-time profiles.

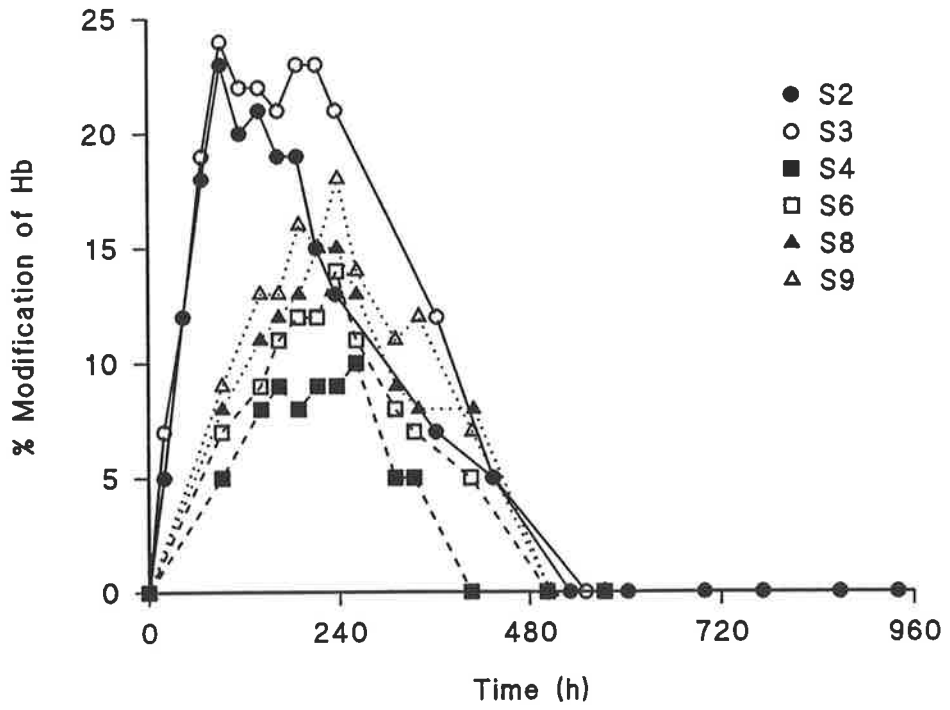


Figure 6.5 Individual %MOD-time profiles.

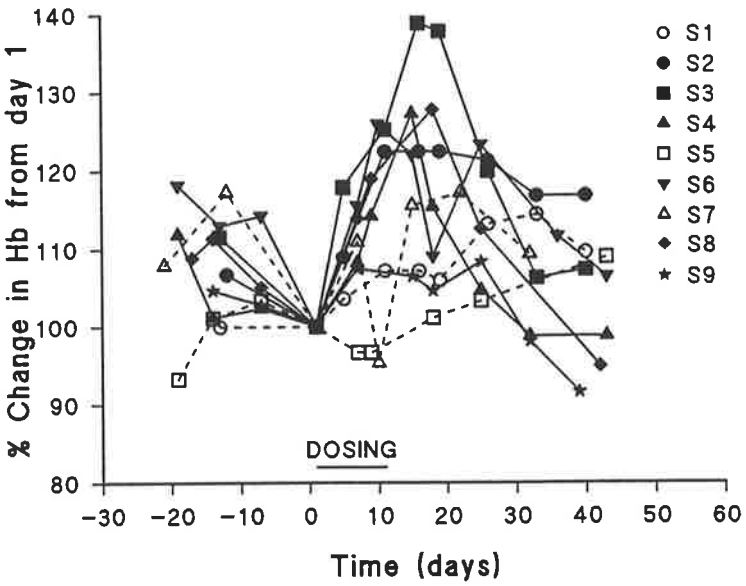


Figure 6.6 Individual Hb levels as % of value on day 1. Subjects receiving placebo are indicated with open symbols.

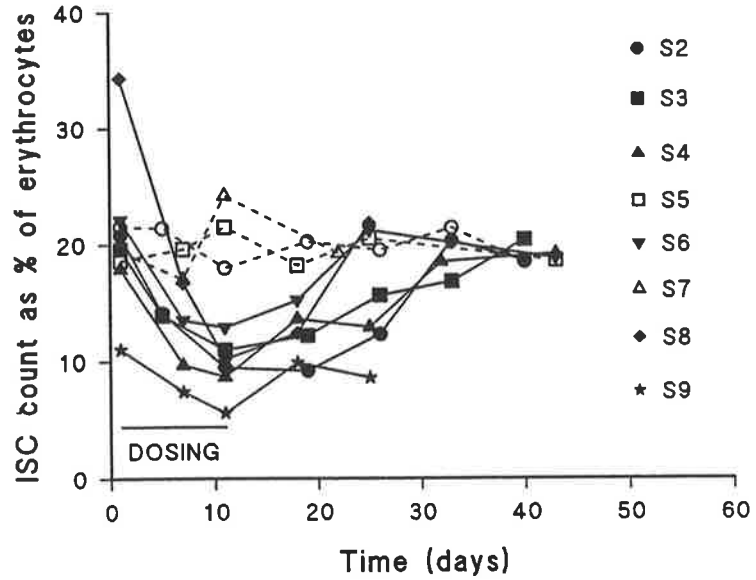


Figure 6.7 Individual changes in irreversibly sickled cell count. Placebo subjects are indicated with open symbols.

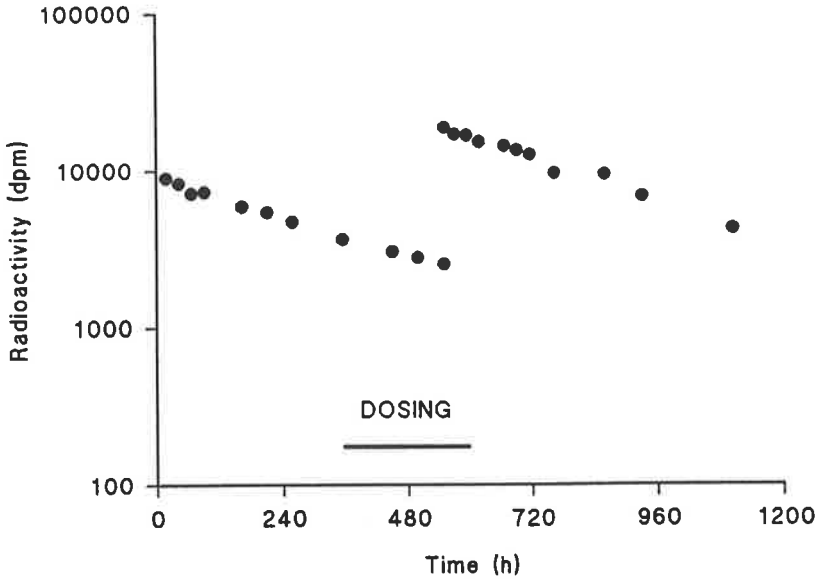


Figure 6.8 Whole blood radioactivity after administration of ^{51}Cr-labelled erythrocytes to Subject 4.

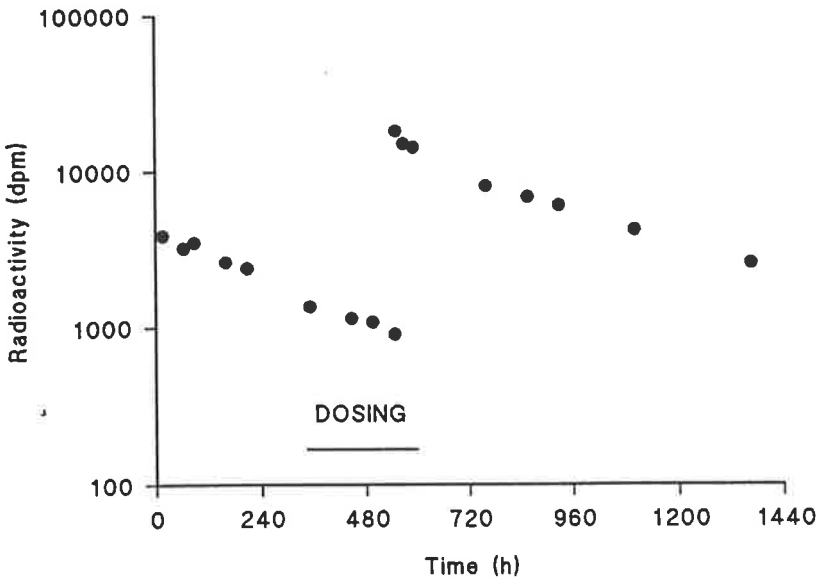


Figure 6.9 Whole blood radioactivity after administration of ^{51}Cr-labelled erythrocytes to Subject 5.

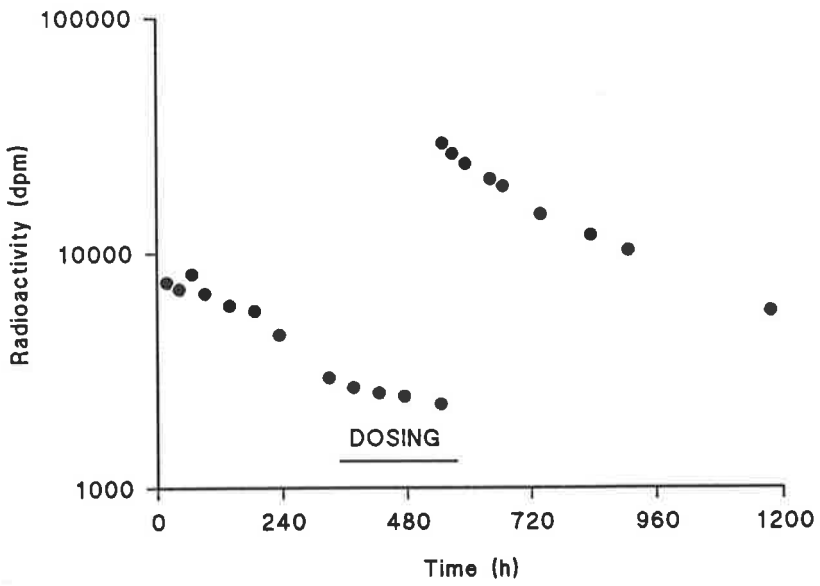


Figure 6.10 Whole blood radioactivity after administration of ^{51}-labelled erythrocytes to Subject 6.

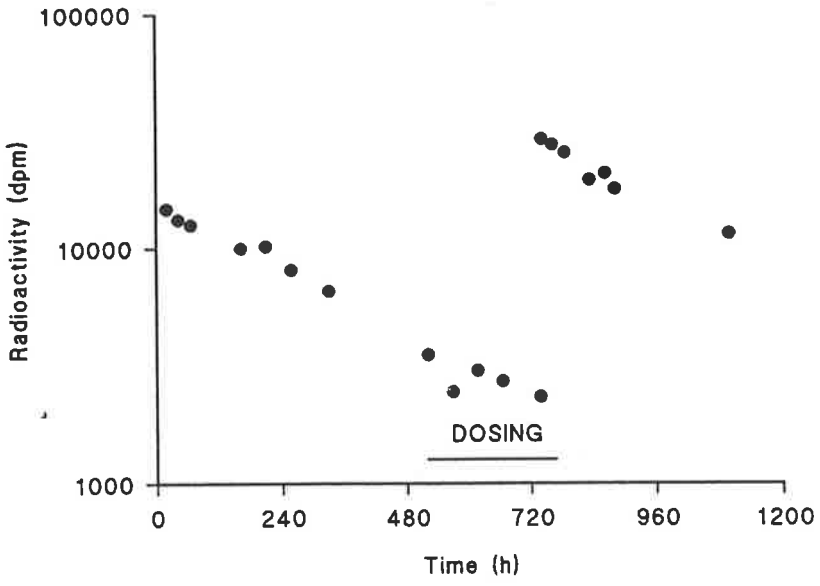


Figure 6.11 Whole blood radioactivity after administration of ^{51}-labelled erythrocytes to Subject 7.

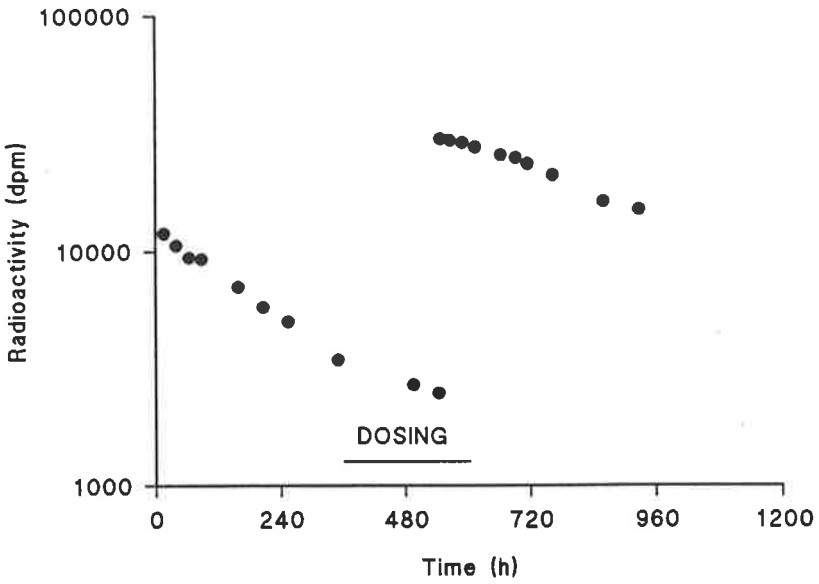


Figure 6.12 Whole blood radioactivity after administration of ^{51}-labelled erythrocytes to Subject 8.

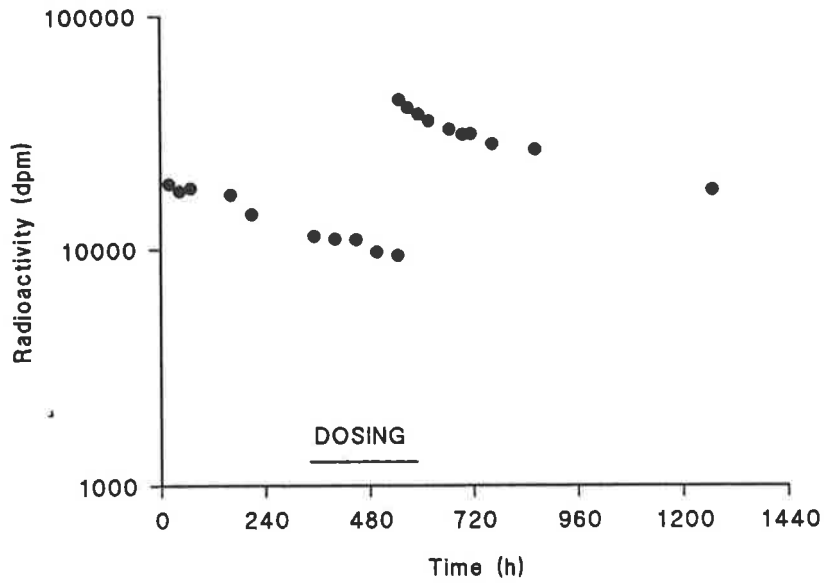


Figure 6.13 Whole blood radioactivity after administration of ^{51}-labelled erythrocytes to Subject 9.

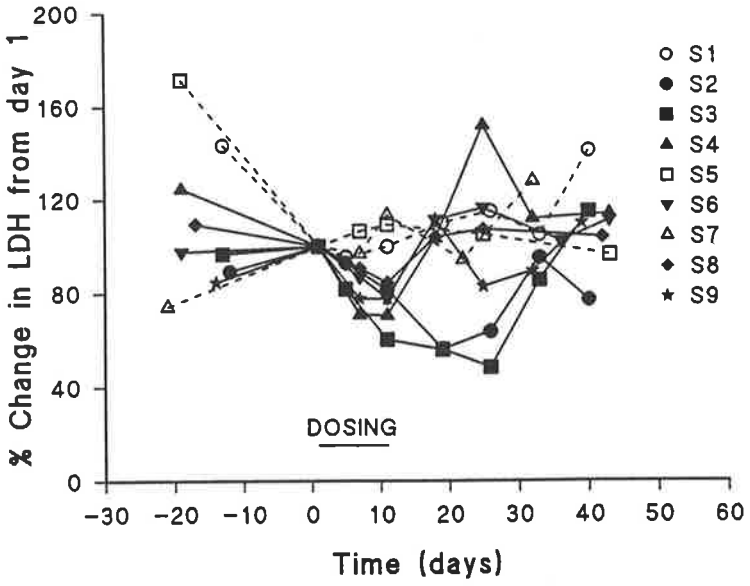


Figure 6.14 Individual LDH levels as % of the value on Day 1. Subjects receiving placebo are indicated with open symbols.

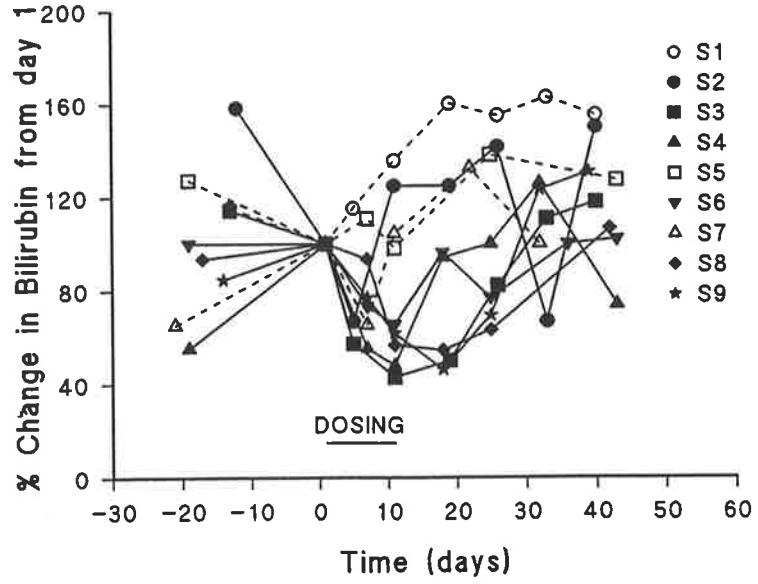


Figure 6.15 Individual levels of plasma bilirubin as % of Day 1 value. Subjects receiving placebo are indicated with open symbols.

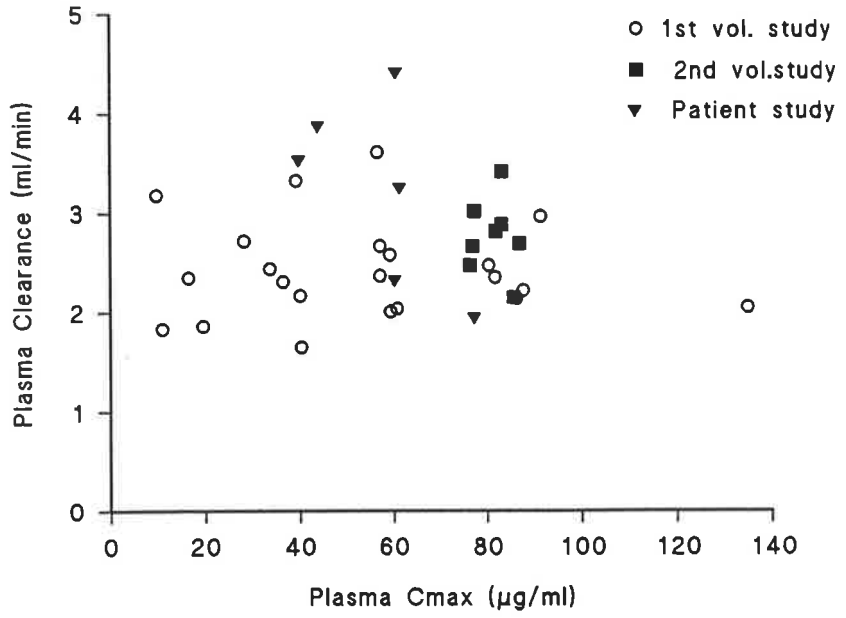


Figure 6.16 Plasma clearance vs C_{max} for all clinical studies with tucaresol.

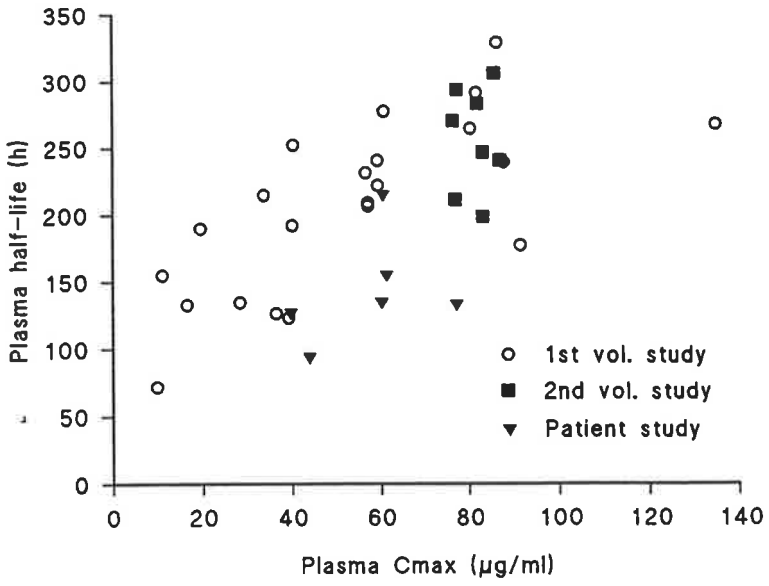


Figure 6.17 Plasma half-life vs plasma C_{max} for all clinical studies with tucaresol.

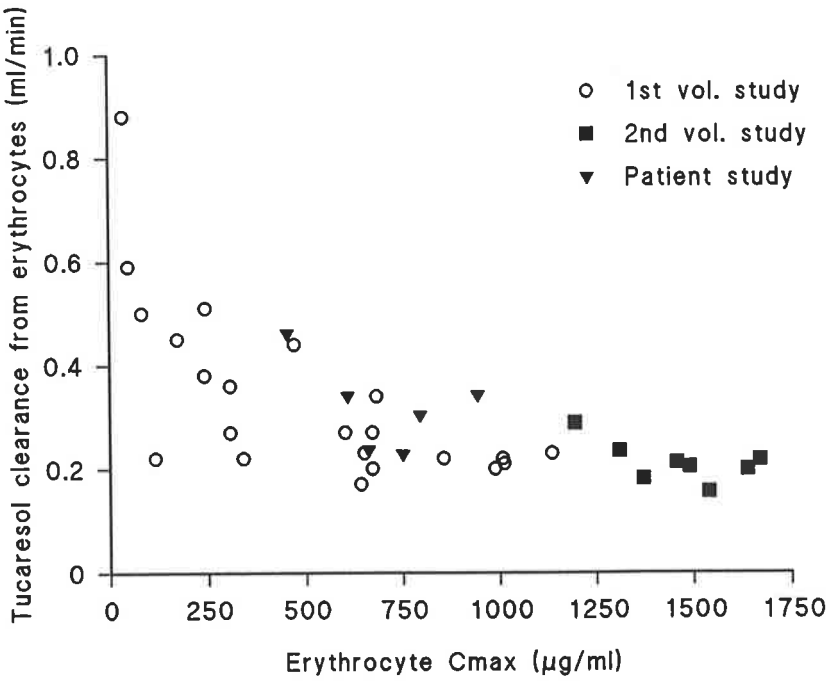


Figure 6.18 Tucaresol erythrocyte clearance vs tucaresol erythrocyte C_{max} for all clinical studies.

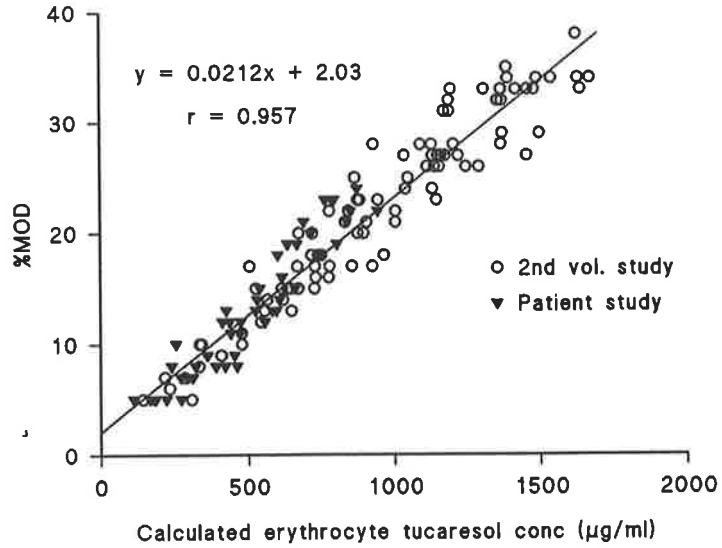


Figure 6.19 Relationships between %MOD and erythrocyte tucaresol concentrations in patients and from the second volunteer study. The regression line is for the volunteer study data.

CHAPTER 7
DISCUSSION

7.1 The exploratory clinical development of tucaresol using a surrogate marker

In this thesis the exploratory clinical development of an unusual and novel compound has been presented. Some of the unusual aspects of the development of tucaresol are discussed below.

Tucaresol is an example of rational drug design. The pathology of the disease it was designed to treat was well understood at the time, as was the structure of its "receptor", the haemoglobin molecule. The knowledge of the quaternary structure of the receptor enabled the design of a molecule with very high affinity for the receptor. The stoichiometric relationship between the drug and receptor, of which the mass in the body could be estimated by routine haematology, enabled a good prediction of the volume of distribution, concentration-effect relationship and hence dose-effect relationship in man before any human exposure. These criteria are rarely fulfilled for a xenobiotic, particularly for one synthesised in 1980 or before.

The good understanding of the pathology of the disease at a molecular level enabled the development of a surrogate marker (%MOD). This pharmacodynamic marker is directly proportional to receptor occupacy and hence we were able to accurately track *in vivo* events occurring at a molecular level. We were confident that %MOD would be useful in guiding dosage regimens. This was important, as, with the relatively narrow therapeutic index, long half-life and large dose size, titration by clinical endpoints alone would have been much slower. Although tucaresol had limited exposure to sickle cell patients, nevertheless this surrogate

marker appears to correlate well with other surrogate markers (haemolysis, ISC's) which are closer to the desired clinical endpoints.

7.2 Future clinical development of tucaresol as a treatment for sickle cell disease

The study in sickle cell patients described in Chapter 6 demonstrated rapid effects of tucaresol on inhibition of sickling. These effects occurred within a few days, paralleling the increasing body drug load. This response is much more rapid than with hydroxyurea, where markers of haemolysis show slow improvement over a time course of several months (Rodgers *et al*, 1990; Charache *et al*, 1992). This is because tucaresol affects all erythrocytes equally and immediately, whereas the effect of hydroxyurea is on the maturation of erythrocytes and requires time for the erythrocyte pool to attain a gradual increase in the proportion of F cells. Furthermore, the achieved levels of HbF with hydroxyurea (mean of 15 $\pm$ 6 % at maximum tolerated dose; Charache *et al*, 1992) is generally less than thought to be required for effective therapy in sickle cell disease (Noguchi *et al*, 1988). In contrast, levels of %MOD greater than 30% can rapidly and reliably achieved in man with tucaresol, and hence from an efficacy viewpoint, tucaresol appears to offer better potential for treatment than hydroxyurea.

However, clinical utility of a drug is not based on efficacy alone but also on tolerability and safety. Hydroxyurea is known to be toxic to the marrow in animals and man and hence its use requires careful monitoring which might make it

unsuitable for use in Third World countries away from laboratory facilities. As a mutagen, there is also concern about carcinogenesis, even though it is generally regarded as having lower carcinogenic potential than many other anticancer drugs. In contrast, the animal toxicology studies with tucaresol did not predict any likely toxicities in man at likely therapeutic dosing levels, with the major effects observed being due to an exaggeration of the desired pharmacological effect, acutely causing poor tissue oxygen delivery and chronically causing stimulation of erythropoiesis. Unfortunately, a stereotyped group of symptoms, comprising fever, tender cervical lymphadenopathy, and a macular rash (in Caucasians) was observed in two studies which was not predicted by the animal studies. The nature of the symptoms, the 7-10 day lag between onset and the start of dosing, and the lack of correlation with dose or plasma concentrations, all suggest an immune mechanism for these adverse effects. Immunologically mediated adverse drugs effects are known to be poorly predicted by animal studies.

Although tucaresol was designed to bind to Hb with high affinity through interaction at a specific site on the molecule, the aldehyde group is likely to form Schiff's base adducts with other proteins at any accessible amino group (terminal amino or lysine residue), and hence tucaresol could act as a hapten. Evidence suggesting very strong binding to plasma proteins comes from the difference in plasma and erythrocyte elimination half-lives, which mis likely to be due to very slow dissociation of drug from a plasma protein-tucaresol complex or possibly due to turnover of a plasma protein-tucaresol complex. This could be examined specifically by incubating

radiolabelled drug with plasma and then separating the proteins by electrophoresis. This would not only indicate to which plasma protein(s) tucaresol binds, but by comparing the electrophoretic pattern with that of plasma not incubated with tucaresol, it may be possible to detect the chemical modification of a plasma protein, which may be relevant to the adverse effects observed.

After the completion of the three clinical studies described in this thesis, tucaresol was found to have immunostimulating properties which are discussed in detail below. These properties suggest that the adverse experiences observed were not idiosyncratic but were related to an unsuspected well-defined pharmacological action with potential applications in clinical conditions other than sickle cell disease. Whether tucaresol might still be a useful therapy for its original intended indication will depend on whether the adverse effects of fever, lymphadenopathy and rash can be avoided or managed either by a different dosing strategy or by other drugs. Further clinical activities in this area, however, will probably depend on the results of further planned laboratory and clinical investigation of the immunological effects of tucaresol.

7.3 Tucaresol as an immunopotentiator

The interaction between antigen-presenting cells (APCs) and helper T cells is a key step in the T cell arm of the immune response. Initially, foreign antigens are internalised in APCs where they are enzymatically degraded into peptides and then displayed on the surface of APCs in a stable complex with class II major

histocompatibility molecules (MHC) (Panayi, 1994). $CD4^+$ T helper cells (Th) have a specific receptor (TCR) which can bind with an antigen/MHC epitope (Panayi, 1994). Formation of this ternary complex (TCR + antigen + class II MHC) causes Th cells to proliferate and produce lymphokines which stimulate cytotoxic T cells, B cells and other cells involved in the immune response (Hamblin, 1994). Efficient activation of Th cells is critical to mounting an effective cell-mediated immune response to foreign antigens. However the formation of the ternary complex alone is insufficient to activate Th cells, and in the absence of other signals, formation of the ternary complex may lead to T cell anergy rather than activation. The major costimulatory signal is provided by intercellular binding between surface glycoproteins (adhesion molecules) found on Th cells and APCs. Rhodes *et al*, had found that this binding was via the formation of a Schiff's base interaction, and that agents known to generate Schiff's base formation are potentiators of antigen-specific Th cells both *in vitro* and *in vivo* (Rhodes, 1989; Gao and Rhodes, 1990; Rhodes, 1990; Zheng *et al*, 1992; Rhodes *et al*, 1994). By chance, Rhodes, who was working at The Wellcome Foundation, overheard a conversation in which Dr R Wootton, (a coinvestigator of the author) was discussing the adverse effects of tucaresol, mentioning that "it did something to the immune system". Rhodes was aware that tucaresol formed Schiff's base adducts with proteins, and hypothesised that the adverse effects of tucaresol observed in the two multiple dose studies were due to T cell activation and immune stimulation. Rhodes, Wootton and the author discussed the results of the human studies with tucaresol. Rhodes then embarked

upon a series of studies investigating possible immunological effects of tucaresol. These studies are summarised below.

7.3.1 Immunological effects of tucaresol *in vitro*

Tucaresol substantially enhanced T-cell proliferation when incubated with normal human peripheral blood mononuclear cells and tetanus toxoid with the optimum response at 0.5 mM. T-cell proliferation to anti-CD3 human monoclonal antibody was enhanced with a 12-fold increase in interleukin-2 production by tucaresol, with the optimum concentration being 0.3 mM. T-cell proliferation in a mixed leucocyte culture from two healthy donors was increased by tucaresol with the optimum concentration being 0.5 mM.

7.3.2 Immunological effects of tucaresol *in vivo*

Mice were immunised locally by sc injection of a peptide antigen (nucleoprotein influenza peptide 343) either alone or with conventional adjuvant (alum or saponin) or tucaresol (1 mg). Antigen-specific $CD4^+$ T helper cell proliferation in cells from regional inguinal lymph nodes was enhanced by tucaresol in a manner comparable to the conventional adjuvant saponin. Intraperitoneal administration of tucaresol to mice enhanced T cell priming to sc immunisation with a protein antigen. A bell-shaped dose-response curve was observed, with the optimum response at 10-20 mg/kg.

7.3.3 Antiviral effects of tucaresol

No effect of tucaresol was observed on the HIV-induced cytopathic effect in a human T cell lymphotropic virus type-1 transformed cell line MT4 *in vitro*. *In vivo*, tucaresol at 10 mg/kg/day for 7 days produced a 1.5 log reduction of cytomegalovirus yield in acutely infected mice, compared to the control group. However, this effect was not observed at 50 mg/kg. Effects of tucaresol in other viral infections in other species are ongoing at the time of writing of this thesis.

7.3.4 Antitumour effects of tucaresol

The effect of tucaresol on a murine adenocarcinoma model was examined. Tumour implants were allowed to establish themselves at the sc administration site in syngeneic mice. After 5 days, mice received tucaresol 10 or 50 mg/kg ip on alternate days for 14 days or no treatment. In the 10 mg/kg group, median tumour weight was decreased 70-80% and in the 50 mg/kg group the decrease was 50% compared to untreated animals. No significant inhibition was observed in nude (T-cell deficient) mice treated with tucaresol, consistent with the effect of tucaresol being mediated by T-cells.

7.3.5 Potential clinical uses for tucaresol as an immunopotentiator

7.3.5.1 Chronic viral infections.

Acute viral infection is a strong stimulus for an immune response which usually leads to viral clearance and immunity. The development of chronic viral infection with viral persistence requires either specific anergy or suppression of cellular immune responses allowing latency. Chronic viral infections which would be

suitable for assessing whether immunopotentiation might be clinically useful would satisfy the following criteria :

- persistent antigenaemia;
- demonstration that immune-modifying agents affect the disease or that immune mechanisms are defective;
- existence of quantitative immunological and viral markers for outcome assessment;
- a major unmet clinical need.

Two conditions meeting these criteria are chronic infection with Hepatitis B virus (HBV) and Human Immunodeficiency Virus (HIV). Clinical investigation of the effect of tucaresol in these conditions is being implemented at the time of writing of this thesis.

7.3.5.2 Cancer.

Although it has been known for some time that many human tumours carry distinctive cell surface markers, it is not known why the immune system does not remove the tumour in a similar manner to that of an allograft. Attempts to treat cancer immunologically have had only modest success. The only common solid tumour for which immunopotentiation is thought to be beneficial is colon cancer when treated with levamisole, which is of minimal benefit.

7.4 Conclusions

In this thesis the exploratory clinical development of an unusual compound has been presented. The compound was developed through a programme of rational drug design for which there was a high expectation of therapeutic success because the molecular basis of the disease was thought to be well understood. A novel surrogate marker was developed which was based of the pharmacology of the compound. This surrogate marker was useful in the early clinical development and appeared to be a good predictor of effects consistent with the desired properties of the compound. However, despite the probability of clinical efficacy continuing to be high, unexpected adverse effects occurred after multiple dosing which resulted in an interruption of the clinical program for the initial indication. A chance event turned this unfortunate occurrence into a possibility of a major new therapeutic indication for the drug which could meet an important therapeutic need. Hence although rational drug design and development have an important role in drug development in the 1990's, serendipity will probably continue to play a significant role in therapeutic breakthroughs if scientists keep an open mind to unexpected developments.

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APPENDIX A

THE PHARMACOKINETICS, TOLERABILITY AND PHARMACODYNAMICS OF TUCARESOL (589C80; 4[2-FORMYL-2-HYDROXYPHENOXYMETHYL] BENZOIC ACID), A POTENTIAL ANTI-SICKLING AGENT, FOLLOWING ORAL ADMINISTRATION TO HEALTHY SUBJECTS

Rolan, P., Parker, J., Gray, S., Weatherley, B… et al. (1993). The pharmacokinetics, tolerability and pharmacodynamics of tucaresol (589C80; 4[2-formyl-3-hydroxyphenoxymethyl] benzoic acid), a potential anti-sickling agent, following oral administration to healthy subjects. *British Journal of Clinical Pharmacology, 35*(4), 419-425.

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APPENDIX B

PHARMACOKINETICS AND PHARMACODYNAMICS OF TUCARESOL, AN ANTI-SICKLING AGENT, IN HEALTHY VOLUNTEERS

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